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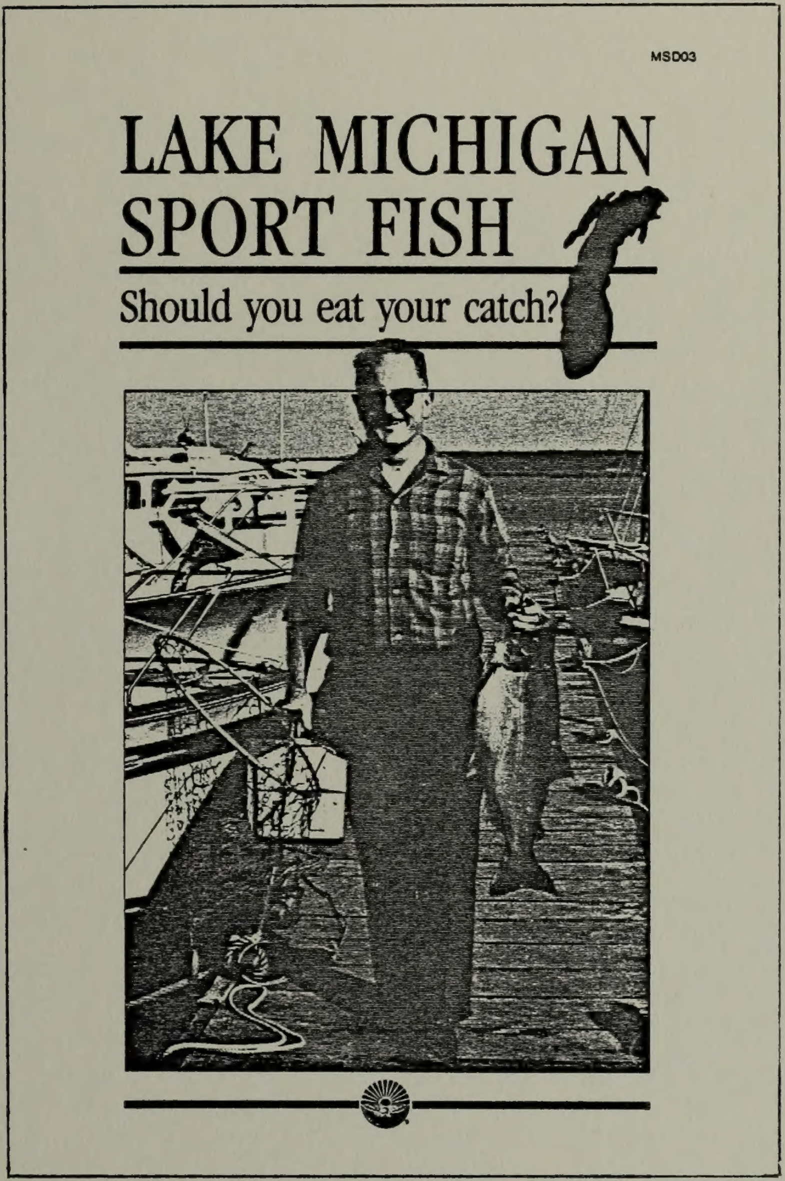

MSD03

LAKE MICHIGAN
SPORT FISH

Should you eat your catch?

August, 1989

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CHAPTER 5

HAZARD ASSESSMENT AND THE CHARACTERIZATION OF CARCINOGENIC AND OTHER RISKS TO HUMANS EXPOSED TO DDT/DDE

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INTRODUCTION

BACKGROUND

Agricultural uses of the pesticide, DDT (dichlorodiphenoltrichloroethane), were banned by the U.S. EPA in 1972 due to the public health hazards posed by its presence in the environment. DDT and its metabolites are persistent in soil, atmosphere, and water, are widely dispersed by erosion, runoff and volatilization, and concentrate in the fat of fish, wildlife, and humans (U.S. EPA, 1980). Table 1 lists some of the relevant properties reported for DDT by the U.S. EPA (U.S. EPA, 1980; U.S. EPA, 1984).

Table 1. Measured Or Calculated Physical Properties of DDT Isomers Summarized by U.S. EPA.

Molecular Weight	354.5
Vapor Pressure at 20°C	1.5×10^{-7} mm Hg for p,p'-DDT 5.5×10^{-6} mm Hg for o,p'-DDT
Water Solubility at 25°C	1.2 - 25 ug/l for p,p'-DDT 26 - 85 ug/l for o,p'-DDT
Log Octanol/Water Partition Coefficient	3.98 - 6.19
BCF	10^3 - 10^6

The manufactured product, technical DDT, is a mixture of isomers and breakdown products. The composition of technical DDT has been reported to be 77.1% p,p'-DDT, 14.9% o,p'-DDT, 0.3% p,p'-DDD, 0.1% o,p'-DDD, 4.0% p,p'-DDE, and 0.1% o,p'-DDE, plus 3.5% unidentified compounds (U.S. EPA, 1980).

HUMAN EXPOSURE

Exposure Routes

Drinking Water:

The intake of DDT was estimated by Hayes (1966) to be 0.04 mg/person/day in food, 4.6×10^{-5} mg/person/day in water, and 9×10^{-6} mg/person/day in air. Therefore, during a period when the use of DDT was legal in the U.S., greater than 99% of human exposure to DDT was via the diet. The U.S. EPA (1980) concluded that dermal, inhalation, and water intake account for less than 10% of the total DDT intake.

The U.S. EPA Ambient Water Quality Criteria Document for DDT reported that surface water DDT concentrations in the Great Lakes had been measured from nondetectable to 0.27 ug/liter (ppb) (U.S. EPA, 1980). This document concluded that:

> ...recorded DDT residues in water probably make only a minor contribution to DDT ingestion by human populations but may contribute to bioconcentration in aquatic species and higher organisms in the food chain.

Rainwater concentrations have ranged from 0.018 to 0.066 ug/liter in both agricultural and nonagricultural areas (WHO, 1984). WHO (1984) concluded that in recent years potable water concentrations were less than 1 ug/liter and average levels were similar to rain water.

Food:

The primary route of human exposure to DDT is from ingestion in the diet. Meat, fish, poultry, and dairy products were reported to be the main dietary classes that were most highly contaminated in 1980 (U.S. EPA, 1980). The overall residue levels in food continually decreased from 1965 to 1973. Estimates of total dietary intake decreased from 62 ug/person/day in 1965 to 8 ug/person/day in 1973. The total DDT equivalent in human tissue for the U.S. population decreased from an average of 8 ppm in 1971 to 5 ppm in 1974.

The WHO (1984) concluded that greater than 90% of the DDT stored in humans is derived from food. Data for adult market basket samples collected and analyzed between October 1980 and March 1982 were reported by Gartrell et al. (1986b). DDT concentrations in all food groups ranged between 0.001 and 0.007 ppm. A person's average daily intake from food was reported to be 0.0387 ug/day. All of the exposure was attributable to leafy and root vegetables. The metabolite, DDE, was measured in more food groups and the average concentration in all groups ranged between trace levels and 0.038 ppm. The average daily intake for DDE was 2.21 ug/day. DDE was identified in dairy products, meat, fish and poultry, potatoes, leafy vegetables, legume vegetables, root vegetables, garden fruits, fruits, oils and fats, and sugar and adjuncts. The highest percent of the average daily intake was accounted for by dairy products (1.05 ug/day, 47.4%) followed by meat, fish and poultry (0.777 ug/day, 35.1%). Leafy vegetables and root vegetables accounted for 6.0% (0.132 ug/day) and 6.6% (0.146 ug/day), respectively.

Pesticide residues in the food of infants (six months old) and toddlers (2 years old) were reported for the same time period (Gartrell et al., 1986a). DDT was not identified in the infant or toddler diet. DDE was found in whole milk, other dairy and dairy substitutes, meat, fish and poultry, potatoes, vegetables, and oils and fats with a range for all food samples in which DDE was detected between trace levels and 0.01 ppm. The average daily intake for all food was reported to be 0.897 ug/day. Sixty-two percent of the daily DDE intake was accounted for by whole milk (0.555 ug/day). Other dairy products accounted for 20% (0.179 ug/day), and meat, fish and poultry accounted for 9.6% (0.0862 ug/day) of the daily DDE intake in infants and toddlers. The daily DDE intake is equivalent to 0.109 ug/kg/day for 8.2 kg infants and 0.092 ug/kg/day for 13.7 kg toddlers.

Atmosphere:

DDT concentrations measured in the atmosphere are very low. Measurements of atmospheric DDT residues in various points in the United States between 1973 and 1975 ranged between 0.01 x 10^{-9} g/m^3 and 0.8 x 10^{-9} g/m^3. In contrast, exposure to workers employed in industries involved in the manufacture or use of DDT was quite significant. The World Health Organization (1984) reported that DDT concentrations in nonagricultural areas has been measured between 0.001 x 10^{-9} to 0.00236 x 10^{-9} g/m^3.

Human Body Burden

DDT Workers and the General Population Compared:

A national summary of total DDT equivalent residues in human adipose tissue reported in 1975 revealed that the geometric mean adipose concentration of total DDT in the total U.S. population decreased from 7.87 ppm in 1970 to 5.89 ppm in 1973 (U.S. EPA, 1980). Long-term storage of DDE was reflected by an increase in the percent of total DDT found as DDE over this period from 77% to 81%.

Mean DDT levels in human milk in 1977 were reported to be 529 ug/kg fat (ppb) (99 percent of 1400 women) with a maximum level of 34,369 ug/kg fat (ppb) (U.S. EPA, 1980). DDE residues were found in 100% of the samples. Mean and maximum levels were 3521 ug/kg fat (ppb) and 214,167 ug/kg fat (ppb). The serum of newborn infants has been found to contain DDT concentrations slightly lower than those of their mothers (O'Leary et al., 1970). This indicates that the placenta presents only a partial barrier to the DDT present in maternal blood.

Studies of occupational exposure to DDT have provided data on the relative levels of DDT in the blood of exposed workers and unexposed controls from the same industry or from the general population. A sample of 25 workers from three insecticide plants directly exposed to DDT had average plasma concentrations of 1.03 ug/ml (ppm), ranging between 0.16 and 3.25 ug/ml (ppm) (Rabello et al., 1975). A sample of 25 indirectly exposed workers from the same plants had an average DDT concentration in plasma of 0.38 ug/ml (ppm), ranging from 0.03 to 1.46 ug/ml (ppm). The average DDT level in another exposed group (8 individuals) from one of the plants was 0.24 ug/ml (ppm) with a range of 0.09 to 0.54 ug/ml (ppm). These levels were compared to 10 control subjects working at the Instituto Butantan with an average DDT plasma concentration of 0.03 ug/ml (ppm) and a range of 0.02 to 0.04 ug/ml (ppm). This study indicated that average plasma DDT levels in workers were 8 to 34 times those in the general population of Brazil.

A study of 35 workers at the Montrose Chemical Corporation who were employed for more than five years with relatively heavy occupational exposure to DDT compared DDT concentrations in serum, fat and urine of the working population to values reported for the general population by Hayes et al. (1965) (Law et al., 1967). The mean values for p,p'-DDT in fat of men in the different exposure groups ranged from 39 to 128 times those reported for the general population (111.85 ± 12.61 ppm exposed vs 1.15 ± 0.22 ppm unexposed). The corresponding mean values for total DDT residue in fat were 12 to 32 times higher (203.74 ± 23.27 ppm exposed vs 8.17 ± 1.14 ppm unexposed). Serum DDT levels were 3 to 10 times higher in the occupational group compared to the general population (0.59 ± 0.072 ppm exposed vs 0.073 vs 0.0013 ppm unexposed). The average DDT concentration in fat was 344 times greater than the average concentration in serum. The mean values for total DDT residue in urine of the workers were 28 to 78 times the mean for the general population (1.25 ± 0.135 ppm exposed vs 0.029 ± 0.0018 ppm unexposed).

Some differences were noted between the two populations. DDE comprised 25% to 63% of the total DDT residue found in the fat of the workers whereas 72% to 92% of the total DDT in the fat of the general population was composed of DDE. DDA was the major excretory product in the urine of occupationally exposed men but was undetected in the urine of the sample taken from the general population. The concentration of p,p'-DDE in urine of workers was only slightly higher than the urinary levels in the general population. This excretory product was stated to be of major importance in the urine of the later group. These differences are thought to have reflected the relative degree of exposure to DDT and DDE in the occupationally exposed group and the general population. Average daily intake of DDT, estimated based on levels in fat, was 18 mg/person/day in the high exposure group, 6.2 mg/person/day in the medium exposure group, and 3.6 mg/person/day in the low exposure group.

WHO (1979) summarized the body burden data for workers engaged in the manufacture, formulation, or use of DDT, and for the general population. The serum concentrations are presented in Tables 2 and 3.

Table 2. Concentrations of DDT and DDE Reported in the Serum of DDT Workers (WHO, 1979).

Reference	DDT (mg/l)	DDE (mg/l)	Total DDT (mg/l)
Laws et al., 1967	0.142 - 0.302	0.145 - 0.272	0.358 - 0.737
Poland et al., 1970	0.573	0.506	
Clifford and Weil, 1972	0.022	0.055	
Morgan and Roan, 1974	0.002 - 0.004	0.026 - 0.222	
Almeida et al., 1974	0.225 - 0.345	0.257 - 0.379	0.504 - 0.681

Table 3. Concentration (mg/liter) of DDT in the Serum of Members of the United States General Population (WHO, 1979).

Reference	p,p'-DDT	p,p'-DDE	Total DDT	DDE as % of Total
Yobs, 1969	0.00335	0.00837	0.0143	78.0
Yobs, 1969	0.00342	0.00927	0.014	73.4
O'Leary et al., 1970		0.0152		
Radomski et al, 1971			0.032	

Highly Exposed Subpopulations:

Kreiss et al. (1981) reported a community-wide study of DDT residues and health effects in Triana, Alabama. A total of 518 persons (43.8% male and 56.2% female) were enrolled in the study. Most of the study participants were black (86.9%) and 36% were aged 18 years and younger. The mean duration of residence for adults was 24.9 years. Most of the participants (83%) lived within 0.8 km of cotton fields and 87.5% of the adults had done farm work.

Ninety-four percent of the adults who had been agricultural workers had worked before use of DDT was banned in agriculture. Median fish consumption was 4.3 fish meals per month before publicity about contamination and fell to 0.3 meals per month after the publicity. A fish caught locally in 1979 had an average of 226 ppm total DDT residue in the edible portion. Of the total residue, 68% were DDD isomers and 29% were DDE isomers.

The mean serum DDT level in the study population was 159.4 ng/mL (ppb) (geometric mean; 76.2 ng/mL (ppb); range, 0.6 to 2,820.5 ng/mL (ppb)). The U.S. geometric mean serum DDT concentration at that time was 15.0 ng/mL in persons aged 12 to 74 years. Twenty-eight percent of the Triana population had serum DDT levels more than ten times higher than the U.S. population. Thirty-three percent had serum DDT levels greater than 500 ng/mL. The adipose-serum ratio in the person with the highest serum DDT levels was 228.

Age was the most powerful predictor of log DDT level (r=.62). The correlation with age remained significant after controlling for all other variables that correlated significantly with log DDT. These were fish consumption, serum triglyceride level, alcohol consumption, race, residence years, sex, and Hollingshead Index. The average proportion of total DDT in the form of o,p'- and p,p'-DDE was 86.7% with a range of 59% to 100%. The proportion increased with DDT concentration. The DDE proportion was also higher in adults who had done farm work and in those who ate less fish. The association of serum DDT level with age was interpreted to indicate the accumulation of DDT residues over time in tissues. Age-corrected log DDT levels in blacks were greater than those in whites and were higher in men than women.

Fish consumption before the publicity about DDT contamination correlated most strongly with DDT levels. When known significant risk factors were accounted for, residential proximity to cotton fields, farm work, spraying of farm chemicals, and domestic use of DDT-containing spray did not correlate with log DDT in multivariate analysis. The geometric mean serum DDT level among breast-fed children was significantly higher than in nonbreast-fed children (55.7 vs 28.8 ng/mL, p=0.01). Parity and cumulative months of breast-feeding to children were associated with log DDT levels in adult women when controlling for age, but duration of breast-feeding did not remain correlated with serum DDT levels when controlling for age and fish consumption. Alcohol consumption and cigarette smoking were positively associated with log DDT independent of the other risk factors. The authors suggested that alcohol and cigarettes may affect the metabolism or excretion of DDT, or the partitioning of DDT residues between serum and adipose tissue.

A comparison of Laws et al. study of occupational exposure to DDT and the Kreiss et al. investigation of DDT residues in an Alabama population indicate that occupational exposure and contaminated fish consumption result in serum DDT levels elevated over the levels in the general U.S. population. The fish consumers in Alabama had serum DDT concentrations almost 4 times lower than those in the DDT workers, however it is difficult to compare chemical concentrations determined in different studies because of the different analytical methods used. While average serum DDT levels in workers were 8 times higher than average DDT levels in the serum of the general population reported by Hayes et al. (1965), the geometric mean serum DDT concentration in the Alabama fish eaters was 5 times higher than the U.S. geometric mean serum concentration at that time.

Information on human tissue concentrations of DDT and metabolites is available from reports of investigations of tissue samples obtained as a result of autopsies conducted in the late 1960's before the agricultural use of DDT was banned. These data should be interpreted to provide a high estimate of human DDT body burden.

Autopsy tissues from two major Honolulu hospitals were obtained from 44 subjects aged between 28 weeks of gestation to 88 years (Casarette et al., 1968). Analysis of the samples by electron capture gas chromatography revealed a large variation in concentration among individuals. Table 4 presents the mean values for DDT, DDE and DDD for whole tissue and for extracted lipid. The highest values for all the organochlorines monitored, except dieldrin, were found in bone marrow and liver when concentration is expressed as ppm extracted lipid. When the concentration is expressed as ppm of whole tissue, fat samples contained the highest organochlorine values. The total residue values corresponded to the relative amount of lipid in the tissue, except for the brain.

Table 4. Mean Concentrations of DDT, DDE, and DDD in Human Tissue Samples Obtained From Autopsies (Casarette et al., 1968).

	Concentration (ppm)					
	DDT		DDE		DDD	
Tissue	Whole[1]	Lipid[2]	Whole	Lipid	Whole	Lipid
Perirenal Fat	1.33	3.14	4.64	11.47	0.011	0.02
Mesenteric Fat	1.35	2.85	4.4	10.26	0.047	0.08
Panniculus Fat	1.16	2.81	4.48	9.86	0.018	0.03
Bone Marrow	0.411	4.48	2.08	18.47	0.076	1.45
Liver	0.047	1.75	0.2	12.93	0.033	2.43
TBLN[3]	0.892	3.73	1.38	8.31	0.01	0.03
Adrenal	0.125	0.98	0.875	8.57	0.057	0.94
Kidney	0.827	1.66	0.21	6.32	0.002	0.12
Brain	0.011	0.12	0.083	1.59	0.002	0.22
Gonad	0.015	1.53	0.069	7.78	0.002	0.18
Lung	0.015	1.82	0.059	8.78	0.0009	0.05
Spleen	0.011	2.62	0.031	6.05	0.003	0.49

[1] PPM of whole tissue for residues of DDT, DDE, and DDD detected in 12 different human tissue from 44 autopsies.
[2] PPM extracted lipid for residues of DDT, DDE, and DDD detected in 12 different human tissues from 44 autopsies.
[3] Tracheobronchial lymph nodes.

Hoffman et al. (1967) reported slightly higher concentrations in abdominal wall fat specimens obtained during autopsies of patients who died in four large general hospitals in Chicago. Gas chromatographic analysis of 994 specimens was conducted between 1962 to 1966. Autopsy records were available for 688 of the individuals for whom autopsy specimens were obtained. Some changes in analytical methods were introduced in 1964. Consequently tissue concentration values were reported for 1962 - 1963 (282 samples) and 1964 - 1966 (712 samples). The tissue concentration data are presented in Table 5. DDE plus DDT levels were higher in men than women (10.3 ± 6.7 ppm in men vs 8.8 ± 6.1 ppm in women, Z=2.93). DDE plus DDT levels were also higher in blacks than whites ($Z = -5.27$, $p<0.01$), although the number of samples from blacks was very small (9.8 ± 6.4 ppm in white men vs 17.0 ± 7.2 ppm in black men; 8.3 ± 5.8 ppm in white women vs 12.6 ± 7.0 ppm in black women).

Table 5. Mean Concentrations of DDT, DDE and DDT plus DDE in 994 Human Fat Samples Obtained During Autopsy in Chicago (Hoffman et al., 1967).

Number Cases	Mean Concentration (ppm) DDT	DDE	DDE+DDT	% DDE
1962-1963 282	2.9 ± 2.4	7.4 ± 5.3	10.3 ± 7.2	71.9
1964 1966 712	2.4 ± 2.1	6.8 ± 4.8	9.2 ± 6.8	73.9
Total 994	2.6 ± 2.2	7.0 ± 5.0	9.6 ± 7.0	72.9
959*	2.4 ± 1.7	6.4 ± 3.7	8.8 ± 5.5	72.7
Range	0 - 19.0	0 - 56.1	0 - 64.1	

* Concentration after omission of 35 values greater than the mean plus two standard deviations.

Conclusion

Several important observations can be made based on the study of human exposure to DDT.

1. Food is the most important source of DDE for human exposure, primarily meat, fish and poultry, and dairy products.

2. DDT is transferred to infants in utero and via breast milk.

3. Tissue concentrations of DDT and DDE are higher in blacks than whites.

4. Tissue concentrations of DDT and DDE are higher in men than women.

5. Subgroups with higher exposure are exposed workers and those eating contaminated sport fish.

PHARMACODYNAMICS OF DDT

ABSORPTION

Human

Ingestion of DDT leads to an almost complete absorption of the chemical from the gastrointestinal tract in all species that have been examined. Morgan and Roan (1977), who investigated the behavior of DDT and metabolites in humans, concluded that the uptake and assimilation of DDT in humans is relatively slow and is consistent with a physiologic dependence on intestinal fat absorption. Serum concentrations of DDT and DDE peaked three hours after ingestion of 20 mg technical DDT and then slowly fell to pre-dose levels at the end of 24 hours. The elevated level of serum DDT over the 24 hour period indicated that intestinal absorption progressed at a faster rate than transport out of the vascular compartment into tissue storage. One individual who had ingested a total of 2.82 g technical DDT over 183 days (20 mg/kg/day), had either stored in body tissues or excreted in urine approximately 85% of the ingested dose. Since 95% of dietary fat is absorbed from the intestine, the authors concluded that the absorption of DDT would be similar.

Animal

Fecal excretion of DDT derived radioactivity in Swiss mice measured three days after a high intragastric dose of 250 mg/kg DDT was 59 and 54 percent of the dose in male and female mice respectively (Gingell and Wallcave, 1974). Most of this material was found to be unchanged DDT. This was interpreted by the authors to be a measure of unabsorbed DDT. When 250 ppm DDT was fed in the diet to Swiss albino mice for four months 4.3 - 4.5 % of the ingested DDT was excreted in feces as unchanged DDT. Therefore an absorption efficiency of approximately 95% is indicated when DDT is fed over long periods.

METABOLISM

DDT is metabolized via two separate reductive pathways in mammals producing primary endpoint metabolites, DDE and DDA (U.S. EPA, 1980). The predominant conversion is from DDT to DDD via dechlorination. DDD is then transformed into a series of metabolites which are excreted. DDA is the final metabolite in this series and is excreted either free or conjugated. The other primary pathway occurs via reductive dehydrochlorination resulting in the formation of DDE, which is primarily stored in mammalian tissues but may be excreted to various degrees in different species.

Fawcett et al. (1987) proposed that DDA may also be formed from DDT directly through the production of the aldehyde via a cytochrome P-450-Fe(11)-carbene complex. These authors identified DDA in the excreta of rats treated with DDE and proposed a possible mechanism for conversion through the acid chloride by epoxidation. Although Gold and Brunk (1987) found no urinary metabolites indicating epoxidation of administered DDE, such a metabolite has been identified in the urine of DDT treated mice (Gold et al., 1981). Differences have been demonstrated among rodents in the metabolic and physiologic handling of DDT and metabolites.

The distribution and excretion patterns of DDA, DDD, and DDE in humans led Morgan and Roan (1977) to propose an intermediate role for DDD, rather than DDE, in the synthesis of DDA. Serum concentrations of DDD increased with DDT dosage demonstrating the existence of this pathway in humans. Urinary DDA excretion and the concentration of serum DDD increased with the magnitude of

DDT dosage. In addition, both DDA excretion and serum DDD declined after dosing ended, while DDE concentrations continued an upward trend for months afterward. This information, plus the absence of p,p'-DDA in excess of pre-dosing levels in the urine of two subjects ingesting 5 mg/day DDE for 92 days, indicates that the formation of DDD and DDE occurs by divergent pathways. These investigators concluded that the metabolism of DDT by humans is identical to the pathways reported for mice (Peterson and Robison, 1964) and rats (Jensen et al., 1957; NIOSH, 1978).

DISTRIBUTION

Human

DDT and metabolites have been identified in various body tissues, and pesticide levels correspond to the lipid content of the tissue. One exception is the brain, which accumulates less DDT than expected by its lipid content. (U.S. EPA, 1980). DDT levels in the serum and fat of two human subjects who ingested 10 mg/day or 20 mg/day technical DDT for 183 days increased in proportion to dose. A steady state was reached for o,p'-DDT levels after six months (Morgan and Roan, 1971). In contrast, a steady state was not approached for p,p'-DDT in blood or adipose tissue. The distribution between blood and adipose fat represented a dynamic concentration equilibrium between plasma lipoprotein and tryglyceride in fat cells. Morgan and Roan (1977), using data from human subjects, calculated that the ratio of adipose lipid pesticide concentrations and whole serum pesticide concentrations was 280. Storage efficiency (defined as the maximum concentration during or after dosing compared to the total amount of DDT ingested) of p,p'-DDT in human adipose tissue was estimated to be 63% while that of o,p'-DDT was 33%. The storage efficiency of p,p'-DDE in human adipose tissue was 95%, even greater than that of p,p'-DDT. This indicates that DDE is preferentially stored in human adipose tissue compared to DDT.

DDE levels in fat and serum increased very slowly as a function of time (Morgan and Roan, 1971). Conversion of DDT to DDE occurred after a latency period, and the extent of conversion was low in the dose ranges given (5 - 20 mg/day, Morgan and Roan, 1977). Approximately 10% of the ingested p,p'-DDT accounted for in storage at the end of the six month dosing period was in the form of p,p'-DDE. Curves of p,p'-DDE concentrations in adipose tissue as a function of time (data from Morgan and Roan, 1977 and Hayes et al., 1971) appeared to flatten three to five years after the initial dosing. In contrast, the concentration of p,p-DDE in a group of six subjects dosed 35 mg/day for 183 days continued to increase even after five years (Hayes et al., 1971). Morgan and Roan (1977) estimated that the percent conversion of stored DDT to DDE was probably less than 20% over the three years of observation.

In humans, it was shown that higher levels of DDT and metabolites were present in fetal lipids and in lipids of the placenta and amniotic fluid than in maternal lipids (NIOSH, 1978).

Animal

In exposed CF-1 and Balb/c mice, the highest concentration of DDT and metabolites was found in fat tissue, followed by reproductive organs, liver and kidneys, and then the brain (Tomatis et al., 1971). The most prevalent compound was unaltered p,p'-DDT, except in the liver where p,p'-DDD was present in greatest amount. This was also true for perirenal fat in Swiss mice fed 250 ppm p,p'-DDT for six weeks (Gingell and Wallcave, 1974). Unchanged DDT accounted for 98.7% of the DDT residue present in perirenal fat of male mice while DDD and DDE were present in equal amount at 0.54%. Tomatis et al. (1971) found a direct relationship between the concentration of DDT and each metabolite in each organ and the dose level administered. The mice received dietary concentrations of 2, 20, 50, and 250 ppm technical DDT in a six generation feeding study. Females stored greater levels of metabolites in reproductive organs and fat than males.

Tomatis et al. (1974; 1975) evaluated the storage of DDT and metabolites in the interscapular fat of CF-1 mice exposed to 250 ppm technical DDT in the diet for two different time periods (15 and 30 weeks) and then killed them after a progressively longer recovery period. A calculation of the percent of ingested p,p'-DDT that was measured as p,p'-DDE (ppm in fat) indicates that the CF-1 mouse converts DDT to DDE with greater speed than do humans. Assuming that mice eat 5 grams wet weight feed per day, 250 ppm in the diet is equivalent to 1.25 mg/day. Therefore, a mouse would ingest 131.25 mg and 262.5 mg technical DDT over the course of 15 and 30 weeks respectively. Tomatis et al., (1972) reported that the composition of the experimental compound used in their study was 73 - 78% p,p'-DDT. The ingested p,p'-DDT would have been 102.4 mg and 204.8 mg after 15 and 30 weeks assuming a percent composition of 78% p,p'-DDT. The average p,p'-DDE concentration for males and females after 15 weeks was 41.8 ppm and 71.0 ppm, respectively, resulting in an estimate for the percent DDE formed from the ingested p,p'-DDT of 40.8% in males and 69.3% in females. The conversion of DDT to p,p'-DDE over a period of 15 weeks in mice was greater than a comparable estimate in humans of 10% in six months (Morgan and Roan, 1977).

Table 6 presents the relative proportion of p,p'-DDT, p,p'-DDE and p,p'-DDD present in the fat of treated mice at the end of dosing and after 120 weeks.

Individual variation in tissue concentration was large. The proportion of compounds in fat tissue was similar at the time of killing between groups dosed for 15 and 30 weeks. Although p,p'-DDE and p,p'-DDD comprised a greater percentage of DDT-related material in the fat of these CF-1 mice compared to Swiss mice treated for six weeks (Gingell and Wallcave, 1974), the two metabolites were still present in roughly equivalent quantities at the end of the dosing period. The p,p'-DDT isomer was preferentially stored. At the end of 120 weeks (105 weeks after the end of dosing), the proportion of DDT residue in fat comprised by p,p'-DDD had increased and accounted for more of the ingested dose than p,p'-DDT in males and females treated for 30 weeks. The relative levels of p,p'-DDT became decreased over this time period.

Table 6. Proportion of p,p'-DDT, p,p'-DDE, and p,p'-DDD in Interscapular Fat of CF-1 Mice Treated with 250 ppm Technical DDT.

Treatment	Time of Killing (weeks)	No. Animals		Percent (Average)		
				p,p'-DDT	p,p'-DDE	p,p'-DDD
15 wk DDT	15	M	4	68.2	18.2	10.7
		F	4	79.9	10.7	8.7
	120	M	2	56.3	6.8	34.1
		F	2	41.6	7.7	46.4
30 wk DDT	30	M	4	76.5	10.9	9.2
		F	4	73.6	8.6	10.5
	120	M	2	24.6	19.2	37.5
		F	2	29.1	13.8	36.2

Source: Tomatis et al. (1975).

In contrast to its accumulation in fat, DDT accounted for only 37.5% of the DDT residue in the liver at the end of dosing, while DDD and DDE accounted for 39.3% and 21.4% respectively in Swiss mice fed 250 ppm p,p'-DDT for six weeks

(Gingell and Wallcave, 1974). The proportions of these compounds in the tissues of females were essentially the same as males. Tomatis et al. (1975) found roughly the same results in the livers of CF-1 mice exposed to technical DDT for 15 and 30 weeks, although the relative concentration of DDD after 30 weeks of dosing was higher. In addition, levels of p,p'-DDE were lower than in the Gingell and Wallcave study. DDE content in the livers of hamsters maintained for six weeks on a diet containing 250 ppm p,p'-DDT was much lower, approximately 2% of total DDT residue, and DDD content was higher, about 65% of total residue (Gingell and Wallcave, 1974).

Male Wistar albino rats given i.p. injections of 200 mg/kg body weight DDT accumulated 10% and 6% of the total dose in skin and fat, respectively, after 24 days (Fawcett et al., 1987). Approximately 5% of the dose was measured in visera which was composed of all the major organs and the gut. The majority of a similar dose of DDE was also found in skin and fat (5.4% and 5.3%) after 56 days. A relatively high percent (3.6%) of the DDE dose was also deposited in muscle.

Rats exhibited a distribution pattern of DDT derived material in the liver that was similar to that observed in mice. DDD and DDT were present in equal amounts (58.1 and 57.1 ug) in the livers of male Sprague Dawley rats after 52 days of dosing 14.5 mg/kg body weight p,p'-DDT by gastric intubation, while DDE concentrations were lower (15.9 ug) (Mitjavila et al., 1981). The accumulation rate calculated for DDT-related materials in the liver was equal for DDD and DDT, while less DDE was accumulated and at a slower rate. The rate of accumulation of p,p'-DDD in the carcass was significantly lower than for p,p'-DDE or p,p'-DDT. However, DDT was accumulated in greater amounts in the carcass. DDT accounted for the majority of the DDT residue in the carcass

(77.2%), while DDE and DDD accounted for 9.0% and 13.8%, respectively. DDT accumulation in the perirenal fat of rats increased with dose and was greater in females (Laug et al., 1950). However, the storage ratio of DDT concentration in fat to DDT concentration in diet was higher in the lower dose groups. Males fed dietary levels of 1, 5, 10, and 50 ppm had storage ratios of 21, 12, 8, and 6. Storage ratios in females were 29, 20, 15, and 12.

The accumulation of DDT in Sherman strain white rats also increased with dose (Ortega et al., 1956). Observation of rats fed 5, 15, 50, 200, and 400 ppm technical DDT for 2, 4, or 6 months indicated that a smaller proportion of DDT intake was stored at higher doses than at lower doses. A greater amount was stored in the fat of females. During feeding, the female to male ratio of DDT concentration in fat was 1.5 and 3.0 at dietary levels of 5 ppm and 400 ppm respectively. Equilibrium was reached in all males and in females in the 50 ppm or lower dose groups by four months. Females exposed to higher concentrations had not attained a steady state level by six months.

DDE storage in fat was correlated with increasing dose in females, however this relationship was not established in males. Table 7 presents the proportion of DDT residue in fat comprised by DDE. The percent of total DDT residue in fat that was DDE continued to increase over a 14 month recovery period in both males and females. The percent DDE at the end of the six month dosing period was 10% and 16% in males and females fed a diet of 5 ppm DDT. This proportion varied inversely with dose in males but remained constant in females. By the end of 14 months on the control diet, DDE comprised 35% and 47% in males and females, respectively. The relative amount of DDT related material stored as DDE in females compared to males increased with the duration of exposure to 5.7 at six months of feeding, and then fell to a ratio of 1.5 after the 14 month recovery period.

Table 7. Percentage of Total DDT Related Material Comprised by DDE in the Fat of Rats by Dose Level, Duration of Feeding and Length of Recovery.

		Percent DDE								
		Months of Diet			Months of Recovery					
DDT ppm	Sex	2	4	6	0.5	1	2.5	5	8	14
< 0.6	M	22	7	5	20	13	27	32	18	36
	F	29	18	26	24	17	25	45	50	39
5	M	8	6	10	6	7	21	21	25	35
	F	14	7	16	13	18	44	27	30	47
15	M	6	2	4	3	7	13	26	29	37
	F	15	5	16	17	13	17	32	42	45
50	M	6	0	0	7	5	7	14	32	-
	F	4	7	4	15	18	18	15	39	35
200	M	0	0	0	2	0	4	14	21	41
	F	10	5	22	7	24	31	27	34	61
400	M	1	1	1	0	8	3	15	19	28
	F	10	2	21	28	20	31	33	40	-
Mean of Treated	M	6	2	3	3	5	9	16	22	30
	F	11	5	17	16	20	28	28	40	46
Ratio of Means	F:M	1.8	2.5	5.7	5.3	4.0	3.1	1.8	1.8	1.5

Source: Ortega et al., 1956.

No DDE was detected in the fat of rhesus monkeys dosed with levels from 0.25 to 10 mg/kg/day technical DDT in the diet over 7.5 years (Durham et al., 1963). A steady state level of DDT was measured in fat after the first six months. The capacity to store dietary DDE was demonstrated in rhesus monkeys indicating an inability to convert DDT to DDE.

DDT, DDE and DDD cross the placenta in a number of animal species and are excreted in milk (NIOSH, 1978). In mice, the concentration of metabolites in fetal litter samples was directly related to the DDT dose fed to the mother (Tomatis et al., 1971). The two major DDT residues present in fetuses and placentas were p,p'-DDT and p,p'-DDD. The metabolite, p,p'-DDE accounted for only 5% of the total.

EXCRETION

Human

Storage loss occurred slowly after the discontinuation of dosing in human subjects participating in both the Morgan and Roan (1971, 1977) and Hayes et al. (1971) studies. A proportionately slower rate of loss occurred at lower tissue concentrations. Hayes et al., (1971) reported that after 25.5 months of recovery, DDT storage was reduced to 32% and 35% of the concentrations present at the end of dosing in two groups exposed to 35 mg/person/day, while storage was reduced to 66% of initial concentrations in those given 3.5 mg/person/day. After 37.8 months of recovery, DDT concentrations were 14%, 20% and 26% of the levels at the end of the treatment period in the two high dose groups and the low dose group, respectively. Morgan and Roan (1977) concluded that the decline of p,p'-DDT in adipose fat and blood followed first order decay dynamics. The o,p'-DDT isomer disappeared much faster from fat than the p,p'-DDT isomer after termination of dosing. The loss of p,p'-DDT from adipose storage was stated to be much slower in humans than in the monkey, dog, and rat (Roan et al., 1971).

The decay rate of DDE from human body fat was calculated to be approximately one-eighth that of p,p'-DDT in the range of concentrations measured. Morgan and Roan (1977) calculated the ratio of stored DDE to DDE intake. This value was then compared with the ratio for DDT to test the influence of excretory factors on the relative levels of DDE and DDT in body fat. The ratio for DDE was four times higher than the ratio for DDT (0.295 vs 0.071) suggesting that DDT is more easily eliminated than DDE in humans.

Urinary excretion of DDA has been investigated in the studies of human volunteers by Hayes et al. and Morgan and Roan. DDA excretion in individuals investigated by Hayes et al. increased rapidly after dosing commenced and then more gradually to a maximum level during the first six to 8 months of the 21.5 month exposure period. DDA excretion decreased gradually after dosing stopped but was still above control values 25.5 months after the last dose. A large degree of individual variation was reported. The lowest and highest mean rates of DDA excretion (mg/hr) during the 38th and 94th week of dosing differed by a factor of three for a group of six subjects exposed to 35 mg/day technical DDT (range 0.089 - 0.269 mg/hr). After about 38 weeks, the average proportion of the dosage accounted for by urinary excretion of DDA was 16% and 13% in the high dose (35 mg/day) and low dose (3.5 mg/day) groups, respectively. This observation must be viewed with caution. Although the authors concluded that a steady state had occurred, other reviewers do not agree, particularly with regard to the high dose group.

Morgan and Roan (1977) calculated that the proportion of ingested p,p'-DDT (equivalent) excreted as urinary p,p'-DDA was 12% and 6% in two individuals dosed with 20 and 10 mg/day technical DDT respectively for 183 days and followed for at least three years. The proportion of estimated maximum p,p'-DDT stored in the two subjects excreted as p,p'-DDA was 16% and 8% respectively. The authors were unable to quantitate excretion in subjects dosed 5 mg/day because the elevation in excretion was small and not much larger than baseline DDA excretion values. It was concluded that excretion of p,p'-DDA could not have exceeded 20% of ingested p,p'-DDT in these individuals. The DDE to DDA ratio in urine for those ingesting DDT was 1:700 (Roan et al., 1971). These levels of urinary excretion would account for only a small fraction of the total elimination of DDT in humans determined from tissue decay data.

Excretion of DDE in urine was found to be insignificant in humans suggesting its relative stability in human adipose tissue (Morgan and Roan, 1977). Fractions of one ppb of p,p'-DDE were found in the urine of subjects ingesting 5 mg/day of this metabolite for 92 days. In addition, no excess p,p'-DDA was found in the urine of these individuals.

Animal

The loss of DDT from interscapular fat in mice was substantial after about 12.5 months of recovery from a 30 week treatment period with a dietary concentration of 250 ppm technical DDT (Tomatis et al., 1975). A loss of 98.9% and 99.0% was observed in females and males, respectively. The concentration in the diet of these mice would be equivalent to 1.25 mg/day assuming a food intake of 5 g/day. The average concentrations in the adipose tissue of females after 30 weeks of treatment were 335 ppm p,p'-DDT and 50 ppm p,p'-DDE. After 90 weeks of recovery, the average concentrations of p,p'-DDT and p,p'-DDE in treated females was 0.31 ppm and 0.24 ppm respectively. In males exposed similarly, the average concentrations were 424 ppm p,p'-DDT and 42 ppm p,p'-DDE just after the cessation of dosing and 0.42 ppm p,p'-DDT and 0.19 p,p'-DDE after 90 weeks. Although a large proportion of the stored DDT related residue was lost from fat tissue, the concentration in fat after a 90 month recovery period was still above that in control animals. The levels in controls were 0.014 ppm p,p'-DDT and 0.03 ppm p,p'-DDE.

Rats also demonstrated a large decrease in the concentration of DDT derived residue from tissues over a relatively short period after treatment ended. Male and female Osborne-Mendel rats fed 4 ppm technical DDT in the diet for 20 weeks and allowed to recover for 12 weeks exhibited a 84% and 81% decrease in adipose tissue concentrations of DDT plus metabolites respectively (Datta and Nelson, 1968). In another experiment, male and female rats (strain not specified) fed 50 ppm technical DDT in the diet for 15 weeks experienced a decrease of 80% and 74% DDT from perirenal fat 12 weeks after treatment was discontinued (Laug et al., 1950). The rate of storage loss was proportional to the adipose tissue concentration. Data are presented in Table 8 on DDT concentrations in perirenal fat at the end of dosing and after four weeks of recovery.

Table 8. Reduction in Fat DDT Concentrations After Termination of Treatment and Four Weeks Recovery.

Conc. in Diet ppm		Concentration in Perirenal Fat		
		End of Dosing	4 Week Recovery	Percent Reduction
1	M	26	19	27
	F	32	30	6
5	M	63	43	32
	F	104	81	22
10	M	82	46	44
	F	160	71	56
50	M	284	157	45
	F	588	267	55

Source: Laug et al., 1950

DDA conjugates with glucuronide, glycine and alanine were the primary metabolites identified in the urine of male Swiss albino mice dosed with 250 ug/g technical DDT over four months and in the urine of male Syrian golden hamsters dosed with 250 ug/g over three months (Wallcave et al., 1974). Excretion of DDA in mouse or hamster urine did not change significantly with time. Total DDA excreted as a percentage of ingested DDT was 11.0, 12.1, 10.0, and 8.7 in mice and 6.3, 5.7, and 4.9 in hamsters for successive collection periods of approximately one month each.

DDE was also identified in mouse urine but not in hamster urine. Total DDE excreted as a percentage of ingested DDT was 1.1, 1.8, 3.4, and 5.7 in mice for the same successive collection periods. Excretion of DDE increased significantly with time ($p < 0.005$). The ratio of DDE to total metabolites in urine also increased significantly with time ($p < 0.01$), and at the end of four months almost as much DDE as DDA was found (2.5 mg DDA and 1.65 mg DDE;

days 94 - 121). Feces was also evaluated for metabolites. DDD and unchanged DDT were found in the feces of both mouse and hamster. Excretion of these compounds remained nearly constant over the feeding period. DDD excretion in feces as a percent of ingested DDT was 4.3 and 5.2 in mice and 2.1 and 2.1 in hamsters for collection periods of two months duration respectively. DDT excretion was 4.3 and 4.5 percent of ingested DDT in mice and 1.2 and 1.3 percent in hamsters for the same periods. Polar metabolites were not represented in mouse or hamster feces.

Rats appear to have a greater ability to excrete DDT. Excretion of radioactivity after an intraperitoneal injection of 200 mg/kg body weight purified DDT in male Wistar rats approached 70% over 20 - 25 days (Fawcett et al., 1981). Sixty-four percent of a 200 mg/kg body weight dose of DDE was excreted after 56 days. Excretion of 50% of the radioactivity administered as ^{14}C-DDT occurred in 12.1 days, while excretion of 50% of administered ^{14}C-DDE was accomplished in 23.7 days. The percent of the dose recovered unchanged from the excreta collected during the time required to excrete 50% of the radioactivity, was 0.4 and 5.2, respectively, for DDT and DDE. This would indicate that metabolism of DDE was occurring in the rat.

Jensen et al. (1957) found that 68%, 26%, and 1.3% of the radioactive DDT recovered in a rat maintained for 9 weeks on a diet containing 400 ppm DDT was accounted for by radioassay of the feces, fat, and urine. Radioactivity in feces consisted primarily of DDA conjugates. Bile was found to be the principle source of DDT metabolites occurring in rat feces after intravenous or oral DDT exposure. Sixty-five percent of ^{14}C-DDT administered intravenously to a white rat was collected by cannulation of the bile duct. Only 0.3% and 2% of the dose was found in the feces and the urine in this animal. Large amounts of metabolite were found in the feces of noncannulated rats. Although the majority of metabolites found were DDA-related compounds (89%), small amounts of DDT (8%) and DDE (3%) were also recovered. Since DDA conjugates have not been reported in the feces of mice and hamsters, the authors proposed that these species may be poor biliary excretors compared with the rat. It was suggested that fecal DDD is a product of the conversion of DDT by gut bacteria.

The metabolism and excretion of DDE has been studied in mice. Female 6 to 8 week old Eppley Swiss mice were fed either a control diet or a diet with 250 ppm DDE for 20 weeks. After 20 weeks, both groups (three animals per group) were dosed with 20 mg/kg[C^{14}]DDE in olive oil. Urine and feces were collected over a 72 hour period. Unchanged DDE comprised 95% of the extracted radioactivity in the urine of both control and treated rats. Over 75% of the unchanged DDE was excreted during this first 24 hours. The only identified metabolite, 3′-OH-DDE, was present in only trace amounts in urine. The authors reported that no evidence for the epoxidation of DDE was observed. Less unchanged DDE was excreted in the urine of pretreated mice.

In feces, 58% of the radioactivity extracted was unchanged DDE. The phenol metabolite comprised 28% of the radioactivity. Excretion of the metabolite was highest after a 24 hour delay. The relative amounts in feces were not affected by pretreatment.

Almost twice as much DDE was measured in the livers of DDE pretreated mice, although on a per gram weight basis the levels were the same. The authors noted that a higher level of phenolic products had been identified previously in the excreta of rats and suggested that mice metabolize DDE to a lesser extent.

CONCLUSION

DDT is almost completely absorbed from the gastrointestinal tract of mammals. Absorption occurs relatively slowly at a rate consistent with intestinal fat absorption. Observations on human volunteers (Morgan and Roan, 1977) have indicated that absorption of DDT is similar to that of the experimental animals studied (Gingell and Wallcave, 1974).

DDT is metabolized via two separate pathways in most mammalian species, except rhesus monkeys. Laboratory rodents and humans convert DDT to the two metabolites, DDD and DDE. DDD is then reduced through a series of steps to DDA which is easily excreted. The rhesus monkey apparently does not have the capability to convert DDT to DDE. Differences exist among mammals in the metabolic and physiologic handling of DDT and its metabolites.

DDT levels in the serum and fat of human subjects increased in proportion to dose (Morgan and Roan, 1971). This was also observed in mice (Tomatis et al., 1971) and rats (Laug et al., 1950; Ortega et al., 1956). A steady state was not reached for p,p'-DDT in human volunteers exposed to 10 and 20 mg/day for 183 days (Morgan and Roan, 1977), while rats fed dietary concentrations of 5, 15, 50, 200, and 400 ppm technical DDT for 2 - 6 months reached a steady state in 4 - 6 months (Ortega et al., 1956).

It is apparent from a comparison of DDT storage in the fat of humans (Hayes et al., 1971) with storage in mice (Tomatis et al., 1971), that humans store more

DDT in adipose tissue than mice. Human subjects dosed 0.05 mg/kg/day DDT stored 201.2 ppm over 12.2 months and 280.5 ppm over 21.5 months. Mice fed 0.3 mg/kg/day for 15 - 30 weeks stored 4.6 - 6.7 ppm. This difference may be partly explained by the shorter dosing period in mice.

The storage efficiency of p,p'-DDE in human adipose tissue is greater than that for p,p'-DDT (Morgan and Roan, 1977). Only a small proportion (10 - 20%) of the ingested p,p-DDT was estimated to be converted to p,p-DDE during absorption or from stored DDT over a three year period. Mice appear to metabolize a greater percent of ingested DDT to DDE and at a faster rate than humans (Tomatis et al., 1975).

A high proportion (54 - 80%) of total DDT stored in human adipose tissue is in the form of p,p'-DDE (Hayes et al., 1971; Tomatis et al., 1971) leading Morgan and Roan (1971) to suggest that a large part of stored p,p'-DDE may originate from ingested p,p'-DDE. The distribution of DDT residues follows the same pattern in mice and rats. Mice and rats also store the same proportion of DDT, DDE and DDD in their tissues. Mice and rats fed DDT stored a major proportion of DDT residue in fat as p,p'-DDT (Tomatis et al., 1975; Mitjavila et al., 1981). Humans, occupationally exposed to technical DDT at an estimated level of 0.3 mg/kg/day, stored 34.6% of total DDT in fat as p,p'-DDE whereas the proportion of stored DDE in mice exposed to 0.3 mg/kg/day DDT was 0.4 - 2.7% (see Table 11 in Tomatis et al., 1971).

Female mice store greater amounts of DDT and metabolites in fat and reproductive organs than do males (Tomatis et al., 1971). Female rats were also reported to accumulate larger amounts of DDT and DDE in fat than males (Laug et al., 1950; Ortega et al., 1956). Placental transfer of DDT, DDD and

DDE has been documented in a number of animal species including humans (NIOSH, 1978).

The rate of loss of p,p'-DDT from human fat is proportional to its tissue concentration (Hayes et al., 1971; Morgan and Roan, 1971 and 1977) and was estimated to be slower than in the monkey, dog, and rat (Roan et al., 1971). Table 9 indicates the relative loss of DDT from the fat of human, mouse and rat. The mouse and rat lost a great deal more DDT from adipose tissue than human volunteers over a shorter period of time after dosing was discontinued. Two human subjects receiving 0.5 mg/kg/day for 183 days lost 80% and 86% DDT from adipose tissue in 37.8 months whereas rats dosed 0.18 mg/kg/day for 20 weeks lost a comparable proportion of DDT in three months. Moreover, radioactive DDT in feces accounted for 68% of the radioactivity recovered in feces, urine, and fat of rats fed 400 ppm DDT for nine weeks (Jensen et al., 1957).

Table 9. A Comparison of the Percent Loss of DDT from Adipose Tissue for Various Species.

Species		Dose	Percent Loss from Adipose Tissue			
			Months After Termination of Dosing			
		Mg/Kg/Day	3	12.5	25.5	37.8
Humans[1]		0.5			68, 65	86, 80
		0.05			34	74
Mice[2]		45.0		99		
Rats[3]	M	2.2	80			
	F	2.2	74			
Rats[4]	M	0.18	84			
	F	0.18	81			

Sources:
[1] Hayes et al., 1971
[2] Tomatis et al., 1975
[3] Laug et al., 1950
[4] Datta and Nelson, 1968

The metabolite, DDA, was found in the urine of treated humans (Hayes et al., 1971; Roan et al., 1971) and mice (Wallcave et al., 1974). The excretion of DDA in urine expressed as a percent of dose was also similar for humans (12% and 16%) and mice (8.7 - 12.1%).

Excretion of urinary DDE was insignificant in humans (Morgan and Roan, 1977), while excretion of DDE by mice increased significantly over four months from 1.1% of the ingested dose to 5.7% (Wallcave et al., 1974). At the end of four months, comparable amounts of DDE and DDA were excreted in mouse urine. Humans therefore, appear to be less able to excrete DDE in urine than mice.

Excretion in human feces has not been evaluated. The nonpolar compounds, DDD and unchanged DDT were found in mouse feces (Wallcave et al., 1974). Polar metabolites were not present. In contrast, DDA conjugates were the primary component of the DDT radioactivity measured in the feces of the rat (Jensen et al., 1957). Wallcave et al. (1974) suggested that mice may be poor biliary excretors compared with the rat.

In conclusion, although humans appear to metabolize DDT using the same pathways as laboratory rodents some differences exist. Humans store more DDT and DDE in adipose tissue than do mice. Rodents preferentially store DDT, whereas humans store a greater amount of DDE. Storage loss of DDT and metabolites occurs at a faster rate in mice and rats than in humans. Mice excrete DDE in an increasing proportion over time in the urine, while humans do not excrete significant amounts of DDE in urine. Rodents also exhibit differences. Rats excrete DDA conjugates in feces in contrast to mice, which excrete only DDD and DDT in feces. Hamsters and rhesus monkeys handle DDT differently than do rats and mice.

SYSTEMIC TOXICITY

SYSTEMIC TOXICITY IN HUMANS

The health of human populations exposed to DDT either through environmental or occupational contact has been studied by several investigators. DDT exposure may result in the induction of liver enzymes. A relationship has also been suggested between DDT exposure and an increase in serum triglyceride and cholesterol.

A primarily black population residing in Triana, Alabama was found to have log gamma-glutamyl transpeptidase levels that were correlated with log serum DDT levels independent of age, sex, and alcohol consumption (Kreiss et al., 1981).

Other liver function tests, SGPT and total bilirubin, were not associated with log DDT. Log DDT level explained only 1.8% of the variance in GT activity, and all significant correlates accounted for only 21.4% of the variance. Log triglyceride and cholesterol levels were also associated with log DDT. Log cholesterol correlated with DDT independent of triglyceride level and age, but the addition of either log GT or race to the regression made the contribution of log DDT insignificant. The association of log triglyceride with DDT level controlling for GT and race disappeared when cholesterol was included in the regression. The relationship between DDT, triglyceride, and cholesterol levels is complicated but may reflect an effect of DDT on lipid metabolism.

Poland et al. (1970) studied the effect of prolonged occupational exposure to DDT on drug and steroid metabolism. Study participants were selected from a list of employees at a DDT manufacturing plant who had been employed for more than five years and had received moderate to intense occupational exposure to DDT. The workers were not exposed to other chemicals at the plant. Employees with normal liver function, indicated by normal serum concentrations of bilirubin, alkaline phosphatase, and serum glutamic oxalacetic acid transaminase, and normal renal function, judged by blood urea nitrogen, were admitted to the study. Eighteen male workers with an average duration of employment of 14.4 years were selected. A control population was selected from policemen and firemen in Brownsville, Texas and matched to the study population for age, ethnic origin, smoking habits, alcohol consumption, and medication.

Average serum p,p'-DDT, p,p'-DDE, o,p'-DDT, p,p'-DDD concentrations were 20 to 30 times the concentration reported for the general population in the United States (all DDT-related compounds expressed as p,p'-DDT; control: 51 ± 5 mug/ml (ppb), factory workers: 1359 ± 162 mug/ml (ppb)). In addition, the sum of all DDT-related compounds in the adipose tissue of factory workers was higher than reported for the general population (307 ± 48 ug/gram (ppm)).

Phenylbutazone half-life in the exposed factory workers was significantly reduced compared to the controls (65.5 ± 3.5 hours in workers and 81.0 ± 3.7 hours in controls), and the average urinary excretion of 6B-hydroxycortisol over 24 hours was significantly increased (291 ± 36 ug in workers and 185 ± 17 ug in controls). These parameters are indicators of the induction of liver microsomal enzymes. The serum concentration of DDT-related substances in either the control population or the DDT factory workers did not correlate with the serum half-life of phenylbutazone or the urinary excretion of 6B-hydroxycortisol. The authors suggested that this may be due to genetic differences in susceptibility to enzyme induction in different individuals.

A group of 40 men with extensive and prolonged occupational exposure to DDT in manufacturing or formulating plants were studied by Ortelee (1958). The study group was primarily aged less than 39 years (28 individuals), with 7 men between 40 and 49 years of age, and 5 men above 50 years. The length of exposure ranged between less than one year (2 men) to 8 years, and the group was fairly evenly divided between 1-4 years of exposure (21 men) and 5-8 years of exposure (17 men). An exposure rate was given to each individual based on observation on the job. The highest exposure rate (13 individuals) was estimated to be absorbed doses of approximately 42 mg/man/day. Assuming a body weight of 70 kg, this is equivalent to 0.6 mg/kg/day. Two lower exposure categories were also identified; 15 men exposed to 0.4 mg/kg/day, and 12 men exposed to between 0.14 and 0.28 mg/kg/day. Except for skin irritation, physical, neurological and laboratory findings were within normal ranges, and no correlation between DDT exposure and frequency and distribution of the few abnormalities were seen.

Laws et al. reported two studies of 35 DDT manufacturing workers of which 20 men had high exposure, and 12 men had medium exposure to DDT, respectively (Laws et al., 1967; Laws et al., 1973). The study group had been exposed to DDT for a mean of 15 years, ranging between 11 and 19 years. Medical histories, physical examinations, routine clinical laboratory tests, and chest x-rays revealed no ill effect attributable to DDT exposure. Of the original study group, 31 men were subsequently evaluated for effects on liver function. The mean duration of exposure in the later study was 21 years and ranged between 16 to 25 years. Two of the men had SGPT levels elevated above the normal range, and two men had alkaline phosphatase levels elevated above normal. DDT intake was estimated based on DDT levels in fat or DDA concentration in urine. Dose rates were estimated to be 0.05 mg/kg/day in the three men with low exposure, 0.09 mg/kg/day in the moderate exposure category, and 0.26 mg/kg/day in the high exposure category. These results must be viewed as inconclusive because the study population was very small, and only three men with low exposure were evaluated.

ORGAN-SPECIFIC TOXICITY IN EXPERIMENTAL ANIMALS

Experimental administration of DDT in animals has produced effects in liver, kidney, endocrine system, immune system, and central nervous system, in addition to reproductive and carcinogenic responses. The available literature indicates that the liver and immune system in rodents appear to be most sensitive to long-term, low-level concentrations in the diet.

Liver

Kinoshita et al. (1966) determined that DDT induced rat hepatic microsomal enzymes at dietary concentrations as low as 1 ppm (0.05 mg/kg/day, assuming a ratio of food intake to body weight of 0.05). Groups of 15 male and 15 female Holtzman rats were placed on treated diets at 30 days of age, and a group of 30 males and 30 females was fed a control diet. Concentrations of DDT in the diets were 0.2, 1, 5, 25, and 50 ppm. Groups of treated rats (3 male and 3 female) and controls (6 male and 6 female) were killed after 1, 3, 6, and 13 weeks. Three reactions catalyzed exclusively by microsomal enzymes were evaluated to examine the effects of DDT on microsomal enzyme activity.

A dose-related increase in the rate of oxidative detoxification of O-ethyl O-(4-nitrophenyl) phenylphosphonothioate (EPN), O-demethylation of p-nitroanisole, and N-demethylation of aminopyrine was observed beginning at a dose of 0.05 mg/kg/day. Maximal EPN and O-demethylase activities of approximately 150% of controls were caused by a dietary concentration of 0.05 mg/kg/day and occurred within the first three weeks. The activity then either decreased slightly or leveled off for the next 10 weeks. Maximal N-demethylase activity occurred after one week. Activity then progressively decreased to approximately half the maximal level at 13 weeks. The lowest concentration (0.2 ppm) produced no increase in enzyme activities. The rate of reversal of enzyme induction in rats fed treated diets for 13 weeks and then allowed to recover for four weeks was inversely related to the DDT concentration in the diet. For example, N-demethylase showed the slowest rate of reversal after four weeks with a 23% decrease in activity in rats fed 5 or 25 ppm dietary concentrations and only a 5% decrease in rats fed 50 ppm in the diet.

Females were less sensitive than males to the inductive effects of DDT. Females fed 5 ppm DDT in the diet showed no increased activity for EPN, but did show a slight increase for O-demethylase, and a definite increase for N-demethylase. The results of this study were not evaluated statistically.

A no-effect level of 0.05 mg/kg/day was estimated for enzyme induction in another study which measured the formation of epoxide intermediates from the incubation of aldrin or heptachlor with hepatic microsomes from white rats (an inbred Wistar strain) exposed to dietary concentrations of DDT (Gillett et al., 1966). The rats had been fed 2.5, 10, 25, or 50 ppm DDT in the diet for two to 12 weeks. The epoxidase activity per gram of liver increased with an increasing dose of DDT administered in vivo. A graph of the ratio of the epoxidase activity of control to DDT-treated rats versus the reciprocal of the dietary concentration was presented. The authors estimated that a dietary concentration of 0.9 ppm or 0.05 mg/kg/day would yield a ratio of one indicating a no-effect threshold.

The p,p'-DDT isomer, 100 ppm in the diet for two weeks, produced an increase in specific activity that was twice that produced by the same concentration of DDE. The specific activity induced by this isomer was also 3.5 and 8 times higher than that produced by DDD in the presence of aldrin and heptachlor respectively. This study also observed a difference in response between the sexes. Epoxidase levels in females were less than half that of male litter mates.

Liver weight, as a percentage of body weight, was increased in female Osborne-Mendel rats fed 200 ppm (10 mg/kg/day assuming a food intake of .05 of body weight) for 18 months (Fitzhugh and Nelson, 1947). Increased liver weight became statistically significant in male rats fed a dietary concentration of 400 ppm (20 mg/kg/day). Groups of 12 male and 12 female rats were fed 0, 100, 200, 400, 600 and 800 ppm technical DDT in the diet for 18 to 24 months. Table 10 indicates that in males, the increase in liver weight began to increase at 100 ppm but was not significant. Concentration levels lower than 200 ppm were not tested in the female rats.

TABLE 10. Liver Weights in Rats Fed Dietary Concentrations of Technical DDT for 18 Months (gm per kg body weight).

	Dietary Concentration (ppm)[a]					
Sex	0	100[b]	200	400	600	800
F	32.7		48.7 $p < .01$	42.7 $p < .05$ $- > .01$	67.3 $p < .01$	
M	25.6	32.2	33.2	39.9 $p < .01$	41.4 $p < .01$	47.3 $p < .01$

[a] Diets fed to 12 males and 12 females per dose group for 18 to 24 months.

[b] Diet fed to males only

Source: Fitzhugh and Nelson, 1947.

Overt toxicity was observed in these animals only at higher dose levels. Tremors were reported to occur primarily at dietary concentrations of 600 and 800 ppm DDT. Hyper-irritability, described as a sensitivity of the rats to stimuli and the appearance of fine tremors, developed earlier in females than males. Rats with moderate tremors recovered, however those with severe tremors progressively worsened to the point of convulsions and death. Ten out of 24 animals showed severe tremors prior to death in the 400 ppm dose group and two females fed 200 ppm developed severe tremors. Food intake was not affected by treatment, however a significant reduction in growth was noted at 12 weeks in females fed 400 ppm and in males fed 800 ppm DDT. After 12 months growth was reduced in all treated females, although the reduction at 200 ppm was not statistically significant. Growth in males was affected slightly at 600 ppm and 800 ppm. A dose-related increase in mortality was observed in treated females but was not apparent in males. Mortality in male rats appeared to increase at a concentration of 400 ppm.

Ortega et al. (1956) reported a definite increase in relative liver weight in male and female Sherman strain white rats only at a dietary concentration of 400 ppm and higher. A series of experiments were conducted involving a feeding period of six months and termination of the study at various time points up to 14 months after the discontinuation of treatment. Dietary concentrations were provided at < 0.6 (control), < 0.05 (control), 5, 15, 50, 200, 400, and 1000 ppm technical DDT. Liver weight as a percentage of body weight in males and females dosed with 400 ppm DDT after two months of treatment was 5.2% and 5.3% compared to control values of 3.8%. The dose-relationship was not as obvious at six months, although the value was still elevated in females fed 400 ppm (4.3 treated vs 3.3 control). Use of the bromsulphthalein (BSP) test for altered liver function revealed no significant effect in any of the treated groups at two and four months. Only 4-5 males and 4-5 females were used to derive these measurements (except at 200 ppm which used combined data involving 14 animals per sex) which may account for the lack of a definite dose-response at lower dose levels.

Males and females fed 200 and 400 ppm in the diet were reported to be nervous and hyperactive and experienced severe tremors of short duration. No change in growth rate was reported in rats fed dietary levels of 400 ppm or less. Weight gain data was not presented by the authors.

Organ weight changes were reported in Carworth rats fed 0, 2.5, 12.5, or 25.0 ppm DDT (purity not stated) in their diets for two years (Treon and Cleveland, 1955). Males fed 25 ppm DDT experienced a ratio of liver weight to body weight significantly greater than controls after 18 months or two years. Males in lower dose groups did not develop elevated liver weights. Females fed 2.5 ppm (0.125 mg/kg/day) were reported to have significantly increased liver weights. The data were not presented by the authors, and it was not stated whether significant increases were observed at the higher dose levels in female rats. No significant difference between treated rats and controls was reported for mortality or weight gain.

Long-term studies conducted with mice did not report the results of treatment on liver weight (Tarjan and Kemeny, 1969, Terracini et al., 1973, Tomatis et al., 1972, Ware and Good, 1967). Liver-weight to body-weight ratios were reported to be significantly greater in treated adult male and female beagle dogs than control dogs when the dose groups of 1 and 10 mg/kg/day were compared with controls (Ottoboni et al., 1977). This finding must be qualified by the fact that a comparison between the two dose groups was not significant, and the increased weights were not always related to dose in every generation.

Microscopic alterations have been reported in rodents exposed chronically to DDT at concentrations in the diet lower than those associated with other toxic responses. Fitzhugh and Nelson (1947) reported dose-related microscopic changes which were present with less intensity at the lowest doses tested (100 ppm (males) and 200 ppm (females)). The lesion was described as "hypertrophy and increased cytoplasmic oxyphilia of the centrolobular hepatic cells, plus increased basophilia and margination of cytoplasmic granules, and a tendency to hyalinization of the remainder of the cytoplasm." A centrolobular necrosis or focal necrosis within centrolobular areas superimposed on the typical DDT change occurred in many livers. It was reported to be somewhat less frequent at lower dose levels, and was nonexistent in controls. This damage was reported to be found more frequently in animals found dead rather than in surviving rats.

Slight to moderate damage was described by Nelson et al (1944) in the livers of rats, mice, rabbits and guinea pigs dosed at higher concentrations, by various routes of administration, and for varying time periods. Moderate central low grade necrosis of the liver, and moderate fatty degeneration, low grade central necrosis, a marked excess of sinusoidal macrophages filled with hemosiderin, and slight portal fibrosis was reported in two dogs (male and female) dosed orally with 100 and 50 mg/kg/day DDT.

The lowest concentration in the diet for which pathological changes have been observed in hepatic cells is 5 ppm (0.25 mg/kg/day) fed for a period of 4-6 months (Nelson et al., 1949, Ortega et al., 1956). Ortega et al. reported a very carefully conducted study of DDT treatment in Sherman strain white rats. Groups of eleven males and eleven females were fed diets containing no added DDT, and 5, 15, 50, 200, and 400 ppm technical DDT for six months during which time one animal of each sex was sacrificed at 0, 2, 4, and 6 months. The surviving animals were maintained on the control diet and were sacrificed at 0.5, 1, 2.5, 5, 8, and 14 months. The control diet was occasionally tested for the degree of DDT contamination, and the concentration was found to range up to 0.5 ppm. An additional experiment was conducted using two control groups and one treated group, five animals per sex, exposed to 5 ppm DDT in the diet for six months. One control group of five animals per sex was fed the same diet as in the previous experiment for six months. The second control group, comprised of nine male rats, was fed a rat chow known to contain a DDT concentration of less than 0.05 ppm. This group was fed for only three months. Individuals in all the groups were sacrificed at various points during the feeding period. Samples for histological analysis were taken using methods designed to minimize variation in organ weights and to promote a rapid fixation of tissues. Animals which died spontaneously were not examined for pathological effects because of potential post-mortem changes. Only freshly killed animals were used for histopathology.

Characteristic changes in the liver were described involving neutral and lipoid fat changes, margination, centrolobular cell hypertrophy, and liposphere formation. The neutral and lipoid fatty alteration was described as a nonspecific accumulation of sudanophilic granules within liver cell cytoplasm. It was frequently observed and variable and therefore not suitable for use as an index of tissue damage. Margination of cytoplasmic granules was associated with cell hypertrophy characterized by a mass of finely vacuolated or reticulated cytoplasm. The liposphere, or inclusion body, was said to be characteristic of DDT exposure and was a roughly spherical, complex, cytoplasmic inclusion with a fatty constitution. The structures were observed to increase in number, size, and thickness of the outer capsule with increasing dose. The number of lipospheres per cell increased with dose as did the number of affected hepatic cells.

The proportion of rats responding and the degree of involvement increased with both dose and duration of treatment. In males, one-half and one-third of the rats fed levels of 5 and 15 ppm DDT showed mild changes. Lipospheres were noted in one-half the rats fed 5 ppm DDT at 2-6 months and in one-third the rats fed 15 ppm DDT at 4-6 months. Margination and hypertrophy were reported for a few rats fed 15 ppm at six months. A mild degree of hypertrophy was observed in the hepatic cells of most females fed 200 ppm DDT at four months. Lipospheres were also rarely observed in this dose group. Lipospheres were never observed in the control animals fed the noncontaminated rat chow.

The four changes were all observed to be reversible. Liver cell necrosis was produced only at 1000 ppm after 25 days of treatment. Rats fed 200 and 400 ppm had occasional cells which showed a depletion of the mitochondria, but Golgi apparati were reported to be well developed at all doses. The hepatic changes were further studied using electron microscopy (Ortega, 1962). This evaluation revealed a relative proliferation of endoplasmic reticulum and a peripheral distribution of discrete ribosomes. The inclusion bodies were composed of laminated aggregates of membranes richly supplied with lipid and were closely related to the endoplasmic reticulum.

Hepatic cell alterations characteristic of DDT exposure were also reported in 77 rats fed 5, 10, and 50 ppm DDT for up to 27 weeks (Laug et al., 1950). The severity of effect increased with dose, and males showed more hepatic changes than females. The hepatic changes at 5 ppm were minimal, but according to the authors, "the difference observed between the control and 5 ppm animals represents the smallest detectable morphologic effect of DDT, based on extensive observations of the rat liver as affected by a variety of chemicals." The pathology was conducted without knowledge of exposure status. No hepatic changes were observed in the livers of rats fed 1 ppm DDT.

Other studies of dietary exposure to DDT have reported histopathologic changes in the livers of rats and mice, but not in dogs. Minor, nonspecific degenerative changes in hepatic cells were reported by Treon and Cleveland (1955) in Carworth rats after 18 months or two years exposure to dietary levels as low as 2.5 ppm (0.125 mg/kg/day).

Similar histological alterations were reported in mice fed DDT concentrations as low as 25 ppm (Keplinger et al., 1968) and 100 ppm (Del Pup et al., 1978).

Three studies of DDT effects in mice at lower dose levels (as low as 2 ppm) do not appear to have evaluated organ histopathology (Tarjan and Kemeny, 1969, Terracini et al., 1973, Tomatis et al., 1972). Ottoboni et al. (1977) reported that no pathologic abnormalities attributable to DDT were found in the liver of beagle dogs.

A study of the reproductive effects of DDE exposure noted mild hepatic lesions in 51 treated female Sprague-Dawley rats dosed by gavage with 10 mg/kg/day p,p'-DDE five days per week for five weeks prior to mating and then through gestation and lactation to 8 or 19 days after the birth of the litters (Kornbrust et al., 1986). These lesions were characterized as hepatic swelling, inflammation, increased eosinophilic appearance, increased mitoses, and occasional focal necrosis and hemorrhage.

Kidney

Pathological changes in the kidney have also been reported in experimental animals although to a lesser degree than those observed in the liver. The kidney to body weight ratio was significantly increased in male and female Osborne-Mendel rats fed diets containing 600 ppm technical DDT at 18 months (Fitzhugh and Nelson, 1947). The kidney weights were increased in females at lower dietary levels (200 and 400 ppm), and the small number of rats tested per dose level (less than ten per sex) may have been responsible for the lack of statistical significance. Microscopically, a slight brown pigmentation of the epithelium of the renal convoluted tubules was reported.

Histopathological changes in rat kidney cells were reported at a dose level of 1000 ppm for 18 weeks (Nelson et al., 1944) and for one to six weeks (Ortega et al., 1956). The kidney lesions were described as a moderate number of hyaline renal tubular casts and a few atrophic renal tubules (Nelson et al., 1944), and fatty infiltration of proximal convoluted tubules (Ortega et al., 1956). Ortega et al. noted that kidney changes were not observed in rats fed diets containing 400 ppm or less. Laug et al. (1950) observed no changes in kidney cells of rats fed from 1 to 50 ppm DDT.

Immunological System

Immunological effects have been detected in rats (Gabliks et al., 1975) and rabbits (Street and Sharma, 1975) administered DDT in the diet for short periods of time. Gabliks et al. fed female albino rats (five animals per dose group per test) a semisynthetic diet containing technical grade DDT at 20 and 200 ppm (2.2 and 22 mg/kg/day) for 31 days. Some groups were immunized by two injections of diptheria toxoid and were killed 24 days after the last toxoid injection. Immunized animals fed DDT diets had slightly decreased diptheria serum antitoxin titers compared to immunized control values. Titers in rats fed 20 ppm DDT were 90% of controls, while levels in rats fed 200 ppm DDT were 81% of controls. In addition, live immunized animals fed DDT diets, challenged with intraperitoneal injections of diptheria toxoid to induce anaphylaxis, experienced less severe anaphylactic shock compared to control animals. Rated on a severity scale of 1+ to 4+, rats fed 200 ppm DDT reacted mildly (1+) while controls reacted most severely (4+).

Histamine-containing mast cells, counted in the mesenteries of immunized treated and control rats, were found to be lower in number in the treated groups. The authors concluded that the severity of anaphylaxis was proportional to the number of mast cells in the mesenteries prior to a challenge with tetanus toxoid. The degranulation of mast cells in the mesenteries and subsequent release of histamine was also determined to be correlated with the severity of symptoms. The percent reduction in mast cell number after anaphylactic shock was 41%, 26%, and 6% in the control, 20 ppm, and 200 ppm groups respectively. In addition, DDT inhibited the immunization induced increase in the number of mast cells in the mesenteries indicating that the treated animals were less sensitized to tetanus toxoid. The percent increase in mast cell production due to immunization was 58%, 12%, and no increase in the control, 20 ppm and 200 ppm groups respectively. The authors concluded that DDT releases histamine granules from the mast cells so that fewer histamine containing mast cells remain. Therefore DDT fed animals are less susceptible to anaphylactic shock. Immediate type hypersensitivity reactions may be affected by dietary DDT.

Other treatment-related effects were minimal in this study. Liver-weight to body-weight ratios were slightly increased compared to controls in rats fed 200 ppm DDT. Spleen weight as a percentage of body weight was slightly decreased at this dose level. Treatment had no effect on daily food consumption or body weight gain.

The immune response in male White New Zealand rabbits treated with dietary concentrations of 0, 4, 20, 45, and 150 ppm of the p,p'-DDT isomer for 57 days was evaluated by Street and Sharma (1975). Eight animals per dose group were administered sheep erythrocytes (SRBC) plus Freund's complete adjuvant subcutaneously on day 33. No dose-related change in total and differential leukocyte counts or in hemolysin and hemagglutin titers were observed in response to the SRBC. However, serum gamma globulin values prior to immunization were increased in DDT treated rabbits, and the increase was statistically significant at dose levels of 20 ppm and higher. After immunization, the elevation relative to controls was not significant and was less marked. This observation caused the authors to conclude that a slight degree of immunosuppression was indicated.

The animals were given a tuberculin challenge on days 43 and 56. Response was suppressed significantly at the highest dose level. Scored on a scale of 0 to 4, response in the 150 ppm animals was scored at 0.38 and 0.63, ten and 24 days following the first antigen inoculation. The scores for control animals were 1.00 and 2.71. On the other hand, sensitivity in DDT treated rabbits after the second immunization was reported to be high.

Immune related organs evaluated after 57 days of DDT treatment showed very sensitive responses. The number of cells in the lymph nodes active in producing gamma globulin (indicated by cells binding fluorescein-labelled anti-globulin) was significantly reduced in a dose-related manner beginning at 4 ppm DDT (0.18 mg/kg/day). Germinal center counts in the spleen were reduced significantly in DDT treated animals beginning at 0.18 mg/kg/day. In addition, progressive thymus cell atrophy was observed in treated animals, but the change was not statistically significant. The trend was dose-related except for a very high score in rabbits dosed with the lowest concentration. These results involving immune-related organs indicate that cellular and selected humoral immune responses were decreased in a dose-related manner. The authors noted that the toxic threshold was dependent upon the test used. Those aspects of the immune system that depend on sensitized lymphocytes to carry out cell mediated reactions appeared to be the most sensitive to DDT toxicity. Sensitized lymphocytes originate in the thymus and are found in active centers in the spleen and lymph nodes.

Slight adrenal atrophy and spleen enlargement, significant beginning at 20 ppm (0.92 mg/kg/day), was reported. No gross toxicity or visceral organ pathology was observed, and the authors stated that the animals demonstrated a normal growth rate and food consumption.

Endocrine System

Corticoid metabolism is stimulated by very high doses of o,p'-DDD in laboratory animals and humans (NIOSH, 1978). Dogs are most sensitive to adrenal cortex toxicity. DDT exposure is also associated with the production of moderate colloid depletion in the thyroid at relatively high but sublethal dose levels for short periods of time (Nelson et al.,1944).

The biliary excretion of thyroxine in rats was not affected by DDT exposure, however bile flow and the biliary clearance rate of plasma thyroxine was increased (NIOSH, 1978). The proportion of biliary iodine present as glucuronide was slightly increased and the ratio of bile to plasma was marginally elevated (NIOSH, 1978). The uptake of iodine by the thyroid was not affected.

In another study, 30-75 mg/kg DDT produced marked increases in the thyroid mass and levels of triiodothyronine and thyroxine in the thyroid of rats. The thyroid iodine level was decreased, serum iodine and protein-bound iodine was reduced, serum thyroxine was slightly increased, and serum triiodothyronine and the iodine fraction in the liver was markedly increased (NIOSH, 1978).

Pancreatic tissue has also been shown to be affected by DDT in mice. The examination of isolated pancreatic islets of DDT-exposed mice revealed that an oral dose of 50 mg/kg reduced insulin secretion to 32% of the control value. The islets were also significantly less responsive to tolbutamide, indicating that the pancreatic inhibitory effect of DDT is not specific for glucose stimulation.

Central Nervous System

Several studies have investigated the effect of chronic administration of DDT on target areas of the brain and on behavior and were described by NIOSH (1978). Marked changes in both frequency and amplitude of the EEG pattern of rats given daily doses of 20 mg/kg were observed by Desi et al. (1966) and Farkas et al. (1968) after four weeks. Slight ataxia occurred after five weeks. The cerebellum appears to be particularly sensitive in that DDT-induced changes in cerebellar electrical activity occurred sooner and were greater in magnitude than those observed in other brain areas such as the motor cortex and the reticular formation. The changes were noted before tremors were evident (Woolley and Barron, 1968).

Peterle and Peterle (1971) found that male mice fed 7 ppm technical DDT in the diet were significantly less aggressive than control mice. Treated mice "lost" more bouts determined by posturing and avoidance behavior, and controls made more biting attacks. Tarjan and Kemeny (1969) did not observe changes in spontaneous or caffeine-induced motility in mice exposed to 2.8-3.0 ppm technical DDT in their diet.

Sanyal et al. (1986) studied the biochemical basis for the central nervous system effects of DDT observed in male rhesus monkeys and found that brain lipid metabolism in treated rats was significantly altered. Technical grade

DDT was administered by oral intubation at a dose of 10 mg/kg/day for 100 days. Total lipids were partitioned and evaluated for differences.

The authors reported that no change was observed in the macroscopical appearance of brain or the histological picture of the tissue. However, a significant decrease in total lipid was found and was due to a reduction in cholesterol and phospholipid classes. A significant decrease was noted in the phosphatidyl inositol plus phosphatidyl serine fraction, and the phosphatidyl choline and phosphatidyl ethanolamine fractions ($p<.001$). The sphingomyelin fraction was significantly increased ($p<.001$). Plasmalogen was not affected by treatment. In addition, no effect was observed on the carbohydrate containing lipids and molar ratios. The data was generated from 4-6 observations in each category. The authors concluded that a decrease in phosholipid and cholesterol could lead to a general impairment in the functioning of the central nervous system.

Conclusion

Toxic effects other than carcinogenicity, reproductive toxicity, and teratogenicity have been studied most extensively in rats (Fitzhugh and Nelson, 1947; Nelson et al., 1949; Ortega et al., 1956; Treon and Cleveland, 1955; Kinoshita et al., 1966; Gillett et al., 1966; and Gabliks et al., 1975). Microscopic alterations in hepatic cells and increased activity of hepatic microsomal enzymes have been reported in association with chronic administration of DDT at low dietary concentrations (Nelson et al., 1949; Laug et al., 1950; Ortega et al., 1956; Kinoshita et al., 1966; Gillett et al., 1966). Immunosuppression has also been observed in rats (Gabliks et al., 1975) and rabbits (Street and Sharma, 1975) exposed to relatively low concentrations of DDT.

Mortality and retarded growth appear to be proportional to dose in females but not in males. Mortality in female rats exposed to DDT for 12 months increased with increasing dose beginning at 200 ppm in the diet, the lowest dose tested in females in this study (Fitzhugh and Nelson, 1947). No relation of dose to mortality was observed for males, although some effect was apparent beginning at 400 ppm DDT. Weight gain in females was inversely proportional to DDT dose and became significant between dose levels of 200 ppm and 400 ppm after 12 months exposure. Males did not appear to be affected until dose levels between 400 ppm and 600 ppm, although the decreased weight gains were not statistically significant even at these higher levels.

Central nervous system effects also occurred at higher dose levels in rats, and sex differences in sensitivity were observed. Tremors and hyperirritability were reported by Fitzhugh and Nelson (1947) in females beginning at 200 ppm (severe tremors in two out of 12 exposed) and in males at higher dose levels. Hyperirritability developed earlier in females than males. Ortega et al. (1956) also reported nervousness, hyperactivity and severe tremors of short duration in males and females at dose levels of 200 ppm and 400 ppm.

Peterle and Peterle (1971) reported a decrease in aggression in male mice fed 7 ppm (1.2 mg/kg/day) technical DDT. Other effects on behavior in the offspring of exposed mice have been reported including delays in the acquisition of conditioned avoidance (Al-Hachim and Fink, 1968) and increased errors and length of time to complete a T-maze.

Liver weight, expressed as a percentage of body weight, was increased in rats by DDT exposure. Females responded at a lower dose than males. Treon and Cleveland (1955) reported significantly increased liver weights in males fed 25 ppm (1.25 mg/kg/day) and in females fed 2.5 ppm (0.125 mg/kg/day) for 18 months to two years. The sex difference in sensitivity was also reported by Fitzhugh and Nelson (1947) at higher dose levels.

Histologic alterations in hepatic tissue were observed in both sexes at the low dietary concentration of 5 ppm (0.25 mg/kg/day) for four to six months (Nelson et al., 1949; Laug et al., 1950; Ortega et al., 1956). The lesions increased in severity and intensity with increasing dose but were also reversible. Treon and Cleveland (1955) reported histologic changes in their study of rats exposed to a dose of 2.5 ppm in the diet over 18 months to two years. Although the data were not presented, it is reasonable to assume that effects at a lower dose would be measurable after a longer duration of exposure. No hepatic cell alterations were observed in rats fed 1 ppm (0.05 mg/kg/day) for 27 weeks (Laug et al., 1950).

Hepatic microsomal enzymes were induced in rats at low doses of DDT. Kinoshita et al. (1966) measured significantly increased activity in three microsomal enzymes at a dietary dose level of 1 ppm (0.05 mg/kg/day). Three observations can be made from the results of this study. The threshold response of various hepatic enzymes to increasing DDT concentration is different; the enzyme induction is reversible to varying degrees; and males were more sensitive in responding to DDT exposure than females. The effect of DDT on the hepatic microsomal enzymes was confirmed by Gillett et al. (1966) who determined that the relative potency of DDT-related compounds was in the following order, p,p'-DDT > DDE > DDD. It is interesting that females were more sensitive to the toxic effects of DDT and yet were more resistant to the enzyme inducing properties of the chemical. It is possible that enzyme induction has a detoxifying effect. The increased toxicity in females may also be associated with the finding that females store more DDT and DDE in their tissues than males (Ortega et al., 1956).

DDT exerted an immunosuppressive effect by decreasing the anaphylactic response of rats to a challenge with diphtheria toxoid associated with a reduction in histamine-containing mesentery mast cells. This dose-related effect was significant at the lowest dose tested, 20 ppm (2.2 gm/kg/day) for 31 days. Rabbits were more sensitive to immunosuppressive effects than rats. The immune-related organs in rabbits responded to exposure in a dose-related manner at a concentration in the diet of 4 ppm (0.18 mg/kg/day) over 57 days (Street and Sharma, 1975).

The Ortega et al. (1956) study is appropriate for an analysis of dose-response for liver toxicity in rats because effects were measured at appropriately low doses, study design and methods were carefully discussed, and dietary concentrations including the control diet were periodically monitored. Even in this study sample sizes were small, but pathological alterations were still observed at the lowest dose level used, 0.25 mg/kg/day. It is significant that when a second experiment was conducted using controls and a dose level of only 5 ppm in a separate room, the tissue levels of the controls were 2 to 8 times lower than those in the control rats used in the first experiment with groups exposed to much higher DDT concentrations. The authors noted that this phenomenon was "a measure of the degree of contamination involved in feeding experiments employing large numbers of animals, some of which are maintained on high dosage levels." These results suggest that other experiments may have been less sensitive to subtle effects at lower dose levels. Although the pathological changes reported by Ortega et al. were reversible after exposure was discontinued, fatty changes are considered to indicate cell damage and could be assumed to remain as long as the tissues were in contact with the chemical. Therefore a dose level of 0.25 mg/kg/day could be used as a LOAEL for hepatic toxicity in rats.

A LOAEL of 0.25 mg/kg/day for hepatic cell changes in rats was also reported by Laug et al. (1950). A NOAEL was determined in this study to be 0.05 mg/kg/day. Hepatic microsomal enzyme induction has been reported in rats exposed to this dose level (Kinoshita et al., 1966). Moreover, elevated enzyme activity was indicated in a few DDT manufacturing workers studied by Laws et al. (1973). The men were exposed to an estimated 0.05 mg/kg/day to 0.26 mg/kg/day. Enzyme induction is an indication of exposure to chlorinated hydrocarbons, but it is not clear that this is an adverse effect.

Therefore, the NOAEL for adverse effects on rat liver is concluded to be 0.05 mg/kg/day. A LOAEL of 0.18 mg/kg/day might also be selected based upon the immunosuppressive effects on the lymph nodes and spleen in the rabbit.

REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF DDT

EFFECTS IN HUMANS

DDE in breast milk has been associated with developmental delays in infants (Rogan and Gladen, 1982; Rogan et al., 1986). DDT in human tissue may be involved in the growth of uterine leiomyomas (Saxena et al., 1987).

An epidemiologic study which followed a birth cohort of 900 North Carolina children found that breast feeding did not continue for as long a time in women with higher levels of DDE in breast milk as it did in women with lower levels (Rogan and Gladen, 1982). Women with the lowest levels of DDE (lower 3%) breast-fed for 37 weeks (median), those in the middle 70% breast-fed for 20 weeks (median), and those with the highest DDE levels (upper 3%) breast-fed for 8.5 weeks (median). Women who gave poor weight gain as a reason for stopping were more prevalent in the groups with higher DDE. The median DDE level at birth was 2.44 ppm (fat basis) with the majority between 1 and 7 ppm. Unsuccessful breast feeders were reported to be more prevalent in the groups with higher DDE concentrations. The median PCB level at birth was 1.79 ppm (fat basis) with the majority between 1 and 4 ppm. The observed association of DDE concentration in breast milk with duration of breast feeding was not apparent for PCB concentrations in breast milk.

In a subsequent article, the authors reported the effect of exposure on neonatal measures of toxicity in the cohort (Rogan et al., 1986). No association was found between maternal DDE levels in breast milk and birth weight, head circumference, and hyperbilirubinemia. The association of DDE with abnormalities in the Brazelton Neonatal Behavioral Assessment Scale (BNBAS) was also assessed. The reflex cluster score, specifically those scores indicating hyporeflexia, was associated with DDE exposure. The effect increased with higher maternal body burden levels. This relationship did not change when ten potentially confounding variables were added into the model.

Saxena et al. (1987) measured significantly greater concentrations of total DDT in leiomyomatous tissue from humans than in normal human uterine tissue. Tissue was collected from a sample of 25 women with uterine leiomyomas between

36 and 55 years of age at the time of hysterectomy. Uterine tissue was also collected from a control sample of women of similar age collected at the time of autopsy. Subjects with a known history of accidental or occupational exposure to DDT and other organochlorine pesticides were not included in the sample groups. DDT and metabolites were reported in all the tissue samples. A mean concentration of total DDT of 0.845 ppm with a range of 0.245 to 1.982 ppm was measured in leiomyomatous tissue while a mean concentration of 0.103 ppm with a range of 0.03 to 0.282 ppm was measured in normal uterine tissue. Total DDT was present at seven times higher concentration in leiomyomatous tissue than normal uterine tissue ($p< 0.001$). The authors suggested that DDT, especially o,p'-DDT, may influence the growth of uterine leiomyomas.

EFFECTS IN EXPERIMENTAL ANIMALS

Exposure to DDT in laboratory animals has resulted in reproductive toxicity, embryotoxicity, fetotoxicity, and toxic effects in offspring. Gross malformations have not been reported, although the design of most of the studies in the literature would not have been sensitive to the detection of teratogenic effects. The toxic effects have generally occurred at relatively high dose levels which also caused other target organ effects.

Adverse reproductive effects have been reported in mice (Tomatis et al., 1972, Keplinger et al., 1968, McLachlan and Dixon, 1972, Lundberg and Kihlstrom, 1973, Del Pup et al., 1978, and Schmidt, 1973;Scudder and Richardson, 1970), rats (Jonsson et al., 1975, Treon et al., 1955, Clement and Okey, 1974, Wrenn et al., 1970, and Green, 1969) and dogs (Deichmann et al., 1971). Other studies have reported no reproductive effects due to DDT exposure in mice (Terracini et al., 1973, Tarjan and Kemeny, 1969, and Ware and Good, 1967), rats (Duby et al., 1977), and dogs (Ottoboni et al., 1977). The interpretation of the results of these studies is complicated by varying experimental protocols, the use of different species and strains, and the collection of data on different parameters indicating effects on the parent, fetus, and offspring. In this section, the studies and their results are discussed in light of study designs acceptable to the U.S. Environmental Protection Agency (U.S. EPA) and U.S. Food and Drug Administration (U.S. FDA) (see Methods and Scientific Rationale for Hazard Assessment, Dose-Response Assessment and Risk Characterization). An attempt is made to determine a no observed adverse effect level in each species.

Estrogenic Effects of DDT

Duby et al. (1971) studied the estrogenic activity of technical DDT and observed significantly increased uterine weight six hours after a single i.p. injection of 4 mg/rat. Three daily injections of 2 and 4 mg/rat/day also significantly increased uterine weights. The influence of chronic ingestion (175 days) of 15 ppm technical DDT on the maintenance of uterine weights in ovariectomized rats was also investigated. Nine treated females were ovariectomized 14 days after the weaning of their litters and were killed and necropsied two weeks later. Uterine weights were significantly decreased relative to controls (124.4 mg vs 149.5 mg, $p<0.05$). The authors suggested that circulating uterotropic steroids may have been reduced by the increased catabolism from the induction of hepatic microsomal enzymes.

The uterotropic activity of DDT isomers was demonstrated by Duby et al. (1971) in Sprague-Dawley rats. Uterine weights were significantly increased by 1, 2, and 4 mg/rat o,p'-DDT six and 72 hours after a single injection. Three daily injections of the same dose levels also increased uterine weights. The isomer, p,p'-DDT, caused a significant increase only after three daily injections of 4 mg/rat/day. However, uterine weights were not maintained in ovariectomized females fed 3 ppm o,p'-DDT or 12 ppm p,p'-DDT for up to 175 days.

In a short-term study, Wistar rats (ten/dose level) were treated by gavage with 10 ug or 50 ug o,p'-DDT or p,p'-DDT, or 0.025 ug or 0.1 ug 17-estradiol (Wrenn et al., 1970). The female rats were killed after 15 days of treatment at 33 days of age. Open vaginae were observed in 100%, 90%, and 30% of the rats exposed to the high dose of estradiol, o,p'-DDT, and p,p'-DDT, respectively, compared to 30% of the rats dosed with the vehicle only. The number of "rat-days" the vaginae had been open during the 33 day period was 58, 16, and 12 days in the rats exposed to the high dose level of estradiol, o,p'-DDT, and p,p'-DDT, respectively, compared to 5 "rat-days" in rats exposed to vehicle only. Both DDT isomers had hastened sexual maturity in these rats. Other parameters indicating estrogenic activity including body weight, ovarian, adrenal, and uterine weights, and uterine water content and glycogen content were not affected, however.

A long-term feeding study was conducted by Wrenn et al. (1970) using 0, 1.0, and 2.5 ppm o,p'-DDT from birth through sacrifice of Wistar rats. The rats (288 males and females) were fed the DDT diets for various time periods from the day of birth for up to 168 days. Groups of rats (number per dose group not stated) were killed at 50, 75, 107-112, and 168 days of age. Ovary weights of the 168 day old females fed 2.5 ppm o,p'-DDT were 75 ± 5 mg compared to 59 ± 2 mg in controls. Ovary weights in the lower dose group and the other age groups treated with o,p'-DDT were not significantly different from the controls. No differences in body weight or uterine weight were reported for females. Effects in males were concluded to be inconsistent, but the data were not presented by the authors.

The estrogenic activity of DDT has also been demonstrated by the association of DDT isomers and metabolites with estrogen responsive tumors in rats and humans. The isomer, o,p′-DDT, was found to compete with labelled estradiol for binding to cytoplasmic estrogen receptors from cell cultures derived from both rat uterus and MT2 tumors grown in Wistar-Furth rats (Robison et al., 1985). No competition for binding sites was observed when p,p′-DDD was evaluated. MT2 tumor growth was also supported by o,p′-DDT when estradiol implants were removed from Wistar-Furth rats. Tumor volumes continued to increase in a sample of seven animals when daily injections of 100 mg/kg body weight o,p′-DDT were administered over 20 days. The response was comparable to that produced by the positive controls injected with estradiol. This effect was dose-dependent since 30 mg/kg supported tumor growth in a manner similar to controls injected with corn oil. The isomer, p,p′-DDT, at 100 mg/kg did not support tumor growth, and the authors concluded that the effect of o,p′-DDT was specific.

Reproductive, Prenatal, and Postnatal Toxicity

Rats

Technical grade DDT had a negative effect on fertility in female Sprague-Dawley rats fed 150 ppm (7.5 mg/kg/day) in the diet for 36 weeks prior to mating (Jonsson et al., 1975). Only one out of three females exposed to males in this dose group had vaginal plugs indicating mating and no young were produced. Evidence of mating was observed in three out of five rats fed 75 ppm (3.75 mg/kg/day) compared to 7 out of the 7 matings in control animals. Two of the females in the 75 ppm group bore young, while only three of the control animals produced litters. Litter size was not affected.

Microscopic evaluation of the ovaries revealed follicular cysts in the mature females treated with 150 ppm DDT. Other reproductive parameters including the length of the estrous cycle, and plasma levels of progesterone and 17-hydroxyprogesterone were not affected by DDT treatment.

The authors concluded that the reproductive effects were not secondary to general toxicity because no mortality was observed at eight weeks, and deaths were not significantly greater in treated groups than controls at 36 weeks. Weight was also not significantly different in treated animals than controls. The toxicity data were not presented by the authors, however.

The study design used by Jonsson et al. was unusual in that dosing of the animals began at an older age (148 days of age), and pairing of the animals was not conducted until they were approximately 400 days old. Females were mated with untreated males. Only two dose levels were used, and an extremely small number of rats per dose group were employed to evaluate reproduction. A reduced number of controls bore litters which was attributed to the older age at mating of the rats in this study. Therefore the reduced fertility in the rats fed 75 ppm is difficult to interpret, and it is difficult to concur with the authors conclusion that this dose level had no effect.

Female Osborne-Mendel rats (12 per dose group) fed 200, 400, 600, and 800 ppm technical DDT in their diets over a period of two years presented microscopic changes in their ovaries which were apparent at all dose levels in rats that survived 89 weeks or more (Fitzhugh and Nelson, 1947). The lesions were not found in the ovaries of control animals. They were described by the authors as an "increase in amount of stroma with presence of numerous relatively large, generally irregular pale cells with a moderate amount of cytoplasm."

A three generation study conducted by Treon et al. (1955) over two years reported no reproductive or prenatal toxicity in Carworth rats fed dietary concentrations of 0, 2.5, 12.5, and 25.0 ppm DDT, although pup mortality was increased. The incidence of mortality in pups, 0 to 21 days of age, in the three dose groups was reported to be slight to moderate. These dose levels were reported to have no effect on the number of pregnancies and the number of pups per litter, however no data were presented to confirm this information. The number of animals bred in each dose group was also not reported.

DDT did not cause a dose-related increase in mortality in adult males or females over the two years, and survival and growth were not reduced. A response was noted in the liver at these dose levels. The average ratio of liver weight to body weight was reported to be significantly greater than controls in males fed 25 ppm and in females fed 2.5 ppm. The organ weight data were not presented by the authors. Minor, nonspecific, degenerative changes were reported in hepatic cells.

A decrease in fertility and an increased number of resorptions were reported in mature Sprague-Dawley rats (age not stated) exposed to 7 ppm DDT in the diet for 60 days before mating and for the duration of pregnancy (Green, 1969). In one experiment, pregnant females were sacrificed on day 21, and the embryos were examined using the Wilson method. Resorptions were significantly increased relative to controls (12 resorptions in 15 treated rats compared to 20 resorptions in 41 control rats). The number of embryos was significantly decreased (131 embryos in treated females compared to 396 embryos in control females). The number of abnormal embryos was not significantly affected by DDT treatment, although the dosing period was not specific enough to identify a gross teratogenic effect. In a second experiment, pregnant females were allowed to deliver their litters, which were maintained on the pesticide diet or control diet and bred when mature. Only 6 treated F0 rats out of 25 became pregnant when mated. Seven out of the original 53 pups survived for 21 days. Zero of the five mated F1 females became pregnant. The methods used to statistically evaluate the results were not described.

Quantitative abnormalities in spermatogenesis and fertility were produced in juvenile male rats treated with very high concentrations of DDT (Krause et al., 1975). Groups of 16 Wistar/Han strain male rats, four days old, received 200 mg/kg DDT or 0.1 ml olive oil by stomach tube daily from four to 23 days of age. Two animals per group were killed at age 6, 12, 18, 26, 34, and 50 days. Body weights and various assessments of the testes and spermatogenesis were made. Although body weights were not altered by treatment, testes weight was significantly decreased at day 18 (35 mg vs 72 mg), day 26 (119 mg vs 176 mg), and day 34 (410 mg vs 486 mg), but not day 50 (1042 mg vs 1062 mg). Tubular diameters were significantly reduced compared to controls on days 6, 12, 18, and 26, but approached control values after treatment was terminated. The number of Sertoli cells and a-spermatogonia were significantly reduced during the initial 12 days of exposure, and p-spermatocytes were reduced at days 18 and 26. Spermatids were reduced, but not significantly, on days 34 and 50.

The last four animals of each group were mated on both the 60th and 90th day of life to healthy females. The average number of fetuses and implantations were significantly less in pregnant females mated to treated males than the number in pregnant females mated to controls. When males were mated on day 60, an average of only 0.5 fetuses and implantations were found in the treated group compared to 10.25 fetuses and implantations in controls. The results of the mating at day 90 were similar, with 7.0 compared to 11.75 fetuses and implantations in the treated and control groups, respectively.

Sprague-Dawley rats fed 5 or 15 ppm technical DDT over two generations demonstrated no difference between treated and control animals in growth patterns, time of vaginal opening, fertility or fecundity, litter size at birth, or litter weight at 21 days of age (Duby et al., 1971). The data for these parameters were not presented by the authors, however. Treatment was also reported to have no effect on testicular maturation or fertility in male rats. This study is difficult to evaluate because an insufficient amount of information was given concerning study design. The age of the F0 rats at breeding was not stated, and the number of breeding females was not reported. The F1 breeding sample size was relatively small. Ten females and five males were selected at 25 days of age and mated.

Tests of reproductive toxicity in rats have also been conducted using DDT isomers and metabolites. Wrenn et al. (1970) determined in a two part study that o,p'-DDT has estrogenic activity at high doses and alters ovary weight after long-term exposure to lower dose levels. The results of a long-term feeding study with Wistar rats using dose levels of 0, 1.0, and 2.5 ppm o,p'-DDT were reported.

Effects were measured on the litters of a very small number of rats per dose level (5-7). The data were presented for the total number of pups in the dose group and not for litters thus ignoring potential litter effects on the health of offspring. Therefore, the authors assumed that the chances of survival for each member of a litter was independent. Birth weights of pups in the 2.5 ppm group were significantly higher than controls. This may have been a result of an older (statistically insignificant) age at parturition of dams in this dose group. Pup weights in the 2.5 ppm group were also higher at weaning than controls. The mean number of young born and the mean number of young surviving at weaning was not affected by DDT exposure. Concentrations of o,p'-DDT measured in whole rat milk samples obtained on day 22 of lactation were 0.013 ± 0.003, 0.238 ± 0.049, and 0.403 ± 0.069 ug/g from rats in the control, 1.0, and 2.5 ppm dose group, respectively.

Similar to the lack of response to technical DDT, the chronic ingestion of 4 or 12 ppm p,p'-DDT or 1 or 3 ppm o,p'-DDT over two generations had no influence on growth patterns, time of vaginal opening, fertility or fecundity, litter size at birth, or litter weight at 21 days of age (Duby et al., 1971). The limitations of this report have been discussed previously.

The ortho-para DDT isomer at a concentration of 1000 ppm (50 mg/kg/day) had a negative effect on pup growth, fertility, and fecundity in the F1 generation of Wistar rats in a two generation study (Clement and Okey, 1974). This study was not a typical multigeneration study in that dosing of the F0 rats was not begun until 80 - 100 days of age, and pairs were caged together for six months. Treatment continued through gestation and lactation. The F1 rats were not exposed to DDT other than *in utero* and via nursing. Growth prior to weaning was lower than controls in pups nursing dams exposed to 1000 ppm o,p'-DDT. At weaning, pup weights appear to have been approximately 10% lower than controls. This generation was mated at 105 days of age with untreated males. Data on reproduction were presented for the F1 generation only. Of the four progeny of dams fed 1000 ppm mated, one produced a litter of three live pups, one died during delivery, and two were not fertile. These two females had developed ovarian cysts. All of the females bred in the other dose categories (control, 20 ppm and 200 ppm) produced live litters with similar mean litter sizes. No information was given concerning the health of the F2 pups through weaning. Only one and four females were bred in the 20 and 200 ppm groups, however, with 14 control females mated. Therefore it cannot be concluded that 200 or 20 ppm o,p'-DDT does not cause adverse reproductive or prenatal effects.

The same experimental protocol was followed using 0, 20, 200, or 500 ppm p,p'-DDT. Growth was reported to be severely depressed in pups nursing dams fed 200 or 500 ppm, and all the offspring in the 500 ppm group were dead by 10 days after birth. Postnatal toxicity, therefore, was apparent in F1 pups nursing dams fed 200 ppm p,p'-DDT or greater. The pups in the 200 ppm group that survived to weaning were as large as control animals by 105 days of age when they were bred. All F1 females in the 20 and 200 ppm groups that were bred produced live litters with litter sizes no different than controls. The health of the F2 pups was not followed through weaning which might have revealed toxicity at these lower doses. Again, only 4 and 2 females were bred in the two dose groups respectively. Therefore, it is difficult to conclude that concentrations up to 200 ppm p,p'-DDT are not reproductive or prenatal toxins.

The metabolite, p,p'-DDE, 10 mg/kg/day, was administered to male and female Sprague-Dawley rats by gavage five days per week for five weeks prior to mating, then through gestation and lactation to 8 or 19 days after birth of litters (Kornbrust et al., 1986). This study was conducted to investigate the effects of DDE on neonatal growth and viability and on various indices of lactational performance. Groups of 51 treated and 54 control females were mated at approximately 90 days of age.

DDE treatment did not appear to have an adverse effect on neonatal growth or lactation. No apparent differences were noted between treated and control rats for percent sperm positive, percent pregnant, length of gestation, litter size, or percent female pups. In addition, no effect was found on mammary gland weight, milk production, RNA and DNA content, RNA/DNA, and milk composition. The health of the treated dams was not adversely affected. Maternal body weights and organ weights were not altered. Mild hepatic lesions were noted in treated rats. The lesions were characterized as hepatic swelling, inflammation, increased eosinophilic appearance, increased mitoses, and occasional focal necrosis and hemorrhage. Weight gain measured from lactation day 2 through 19 in 16 DDE exposed litters and 13 control litters was also not altered. The concentration of DDE in milk (ul/ml or ppm) on lactation days 9 and 20 was 24.4 (SE 7.8) and 21.9 (SE 12.4), respectively.

The serum concentrations at the same time points were 5.66 (SE 2.32) and 1.11 (SE 0.34).

Mice

Keplinger et al. (1968) investigated the reproductive toxicity of several pesticides and combinations of pesticides over several generations of Swiss White mice. The study design was generally consistent with the U.S. FDA protocol; four males and 14 females in each dose group were first bred at 120 days of age, and two litters per generation were produced. The age of the F0 animals at first dosing was not reported. The parents in each generation were fed the treated diets through the weaning of the second litter. The dose levels of DDT alone were 25 ppm, 100 ppm, and 250 ppm technical DDT.

Although not evaluated statistically by the authors, the fertility indices of the F0 and F1 generations fed 250 ppm DDT were below the lower limit of the 95% probability range for the mean of the fertility index for the six control groups, except for the first F1 litter. Fertility was not affected by 100 ppm or 25 ppm in any of the six generations. Litter size was not reduced by DDT treatment except in that of the second F2 litter in the 250 ppm group. Other effects were not reported by litter. Therefore, potential litter effects could not be evaluated.

Viability was markedly decreased in the second generation offspring (F2) fed 250 ppm. Sixty-two percent and forty-three percent of the pups born alive died during the first 4 days of life in the two litters. Fatalities were also noted in the dams at this dose level, and no more generations were attempted. Viability was also decreased in the 100 ppm dose group in the third and fifth generations. The lactation index (percent of pups alive at 4 days that were weaned) and the survival index (percent of pups weaned that were alive at 120 days) were reduced in several generations at the 100 ppm dose level as well. The lactation index was slightly decreased in the second, fourth and sixth generations in the 25 ppm group, however no other indices were negatively affected at this dose level. It is possible that other effects may have emerged with more appropriate sample size.

DDT at 25 ppm in combination with 5 ppm aldrin in the diet caused statistically significant decreases in the lactation and survival indices in the second and fourth generations. This effect was more pronounced than the effects of either chemical administered alone at those dose levels. The combination of DDT and chlordane at equal doses of either 25 ppm or 15 ppm also caused significant decreases in the fertility, viability, lactation or survival index in every generation. DDT at 25 ppm only slightly affected lactation and chlordane at 25 ppm affected lactation and fertility only in the last two generations.

Maternal mortality was not discussed for DDT dose levels other than 250 ppm. Pathological changes were reported mainly in the liver for rats in all dose groups and chemical combinations. These were described as fatty metamorphosis with an increased amount of basophilic substances. Hepatic cell necrosis was noted scattered throughout the parenchyma primarily near the central vein. Alterations in lungs, kidneys, and brain were also mentioned. In addition, bronchiolar carcinoma or benign pulmonary adenomatosis was found in 1 or 2 out of ten mice examined in the 25 ppm DDT, 25 ppm toxaphene, or 5 ppm aldrin groups. The combination of chlordane plus DDT was reported to cause more severe damage to the lungs than when they were fed individually. Finally, liver and brain weights were not found to be significantly increased in ten males and ten females examined in each dose group.

Chronic exposure of stable mouse populations to 100 ppm DDT in the diet caused decreased survival in offspring (Del Pup et al., 1978). Four mouse populations maintained at 400 animals (male and female) of all ages were fed the control diet for 120 weeks. The replacement of dead mice was discontinued, and the populations were then fed either the treated or control diet (two populations each) for 70 weeks. Two strains were exposed, random bred albino stock Mil:Ha(ICR) and a four-way cross of C3HeB/FeJ, C57L/J, SWR/J and 129/J. Indices for reproductive parameters were calculated for every five week period for both the cohort born during that period and for the cumulative response up to and including that period. Average population litter indices were adjusted for litter size to avoid the assumption that survival probabilities for each member of a litter were independent. Litter order effect was also accounted for. Only litters with 3 - 11 pups were analyzed to reduce the effects of litter size on mortality. This was an unusual study design which was intended to evaluate the combined impact of chemical treatment with environmental stress.

Fertility indices were not calculated. Viability, defined as survival to four days of age, was not adversely affected. The 100 ppm dose level caused a decrease in the lactation index (survivors at day 30/survivors at day 1) which became significant in the Mil:Ha(ICR) strain at week 20 and in the four-way cross strain at week 30. The reduction in the lactation index was evident throughout succeeding generations. Postweaning survival, however, was better in the treated mice than the controls. It was suggested by the authors that the higher mortality prior to weaning caused the selective survival of DDT-resistant animals. Histopathological evaluations revealed significant changes only in the livers (acute congestion) of the treated four-way cross population.

A dose-related decrease in fertility was not found in CF-1 mice fed 0, 2, 10, 50, or 250 ppm technical DDT in the diet over two generations (Tomatis et al., 1972). Animals were fed the treated diets over their lifetimes, or up to 140 weeks of age. The average number of newborns per litter and preweaning mortality were the only two prenatal and postnatal indicators reported. Offspring in the 250 ppm group had an increased mortality prior to weaning age of 33% compared to 14% in the controls. This data were not presented for each litter. Mortality in the control pups was higher than the other three lower dose groups, however. The data on postnatal effects in these groups are difficult to evaluate because the control diet was reported to be contaminated; F0 control mice accidentally were dosed with 0.79 ppm DDT for six weeks.

Survival was negatively affected in the 250 ppm group beginning in the 60th week. DDT intoxication was reported in several mice primarily after the 70th week in both generations at dietary levels of 250 ppm.

Mortality associated with convulsions was caused by the same dietary dose level (250 ppm) in another two generation study using BALB/c mice (Terracini et al., 1973). The DDT intoxication occurred in both generations as early as 12 weeks of age in males but not until after 80 weeks of age in 14 out of 18 intoxicated females. No convulsions were noted in the lower dose groups, 2 and 20 ppm. No differences between groups was reported for body growth or average weight up to the 80th week of age. Loss of weight in males and females older than 80 weeks of age was observed in the 250 ppm dose group. Five treated males and females were mated in each dose group, not enough to detect more subtle effects. The investigators concluded that breeding performance and pre-weaning mortality were not affected by treatment but did not present the supporting data. The age of animals at breeding was also not stated.

Swiss strain BALB/c mice did not exhibit statistically significant reproductive or prenatal toxicity when mated after 30 days exposure to 7 ppm (0.9 mg/kg/day) technical grade DDT in the diet (Ware and Good, 1967). Postnatal effects were not evaluated. Fertility was lower in the 106 treated pairs (55.45%) than the 100 control pairs (67.0%) but the difference was not statistically significant. Other parameters were not significantly affected including the number of pairs with mortality, fecundity, litters/producing pair, litters/surviving pair, litter size (first and all litters), days to produce (first and all litters), and young produced/day (first and all litters). The test was repeated using BALB/c and CFW strain mice. DDT had a reducing, but not significant influence on the number of litters/surviving pair in BALB/c mice in both tests (0.94 DDT vs 1.19 control, and 1.51 DDT vs 1.68 control). BALB/c mice also had a lower number of litters per producing pair, although the decrease was not statistically significant (1.68 vs 1.91). The CFW strain was not affected.

A report was cited by the U.S. EPA (1984) wherein daily exposure of pregnant mice to 2.5 ppm caused significant embryotoxicity, blastotoxicity, and fetotoxicity (Schmidt, 1973). No teratogenesis was observed.

Behavioral effects were produced in the offspring of CF1 mice treated with very low levels of technical DDT in their drinking water. Behavioral alterations have been demonstrated in DDT-exposed mice and the offspring of exposed parents. Scudder and Richardson (1970) observed a significant decrease in the aggressiveness of male offspring after exposing pregnant CF1 mice and the offspring to 0.01, 0.1, or 1.0 ug/liter technical DDT in their drinking water. The DDT treated water was supplied to the mice beginning at the time of mating, and treatment was continued throughout the experiment. Weanlings, 21 days old, were isolated for two weeks and then were tested for isolate aggression by round robin fighting in aggression cages. Quantitated estimates of aggression were measured as the percentage of animals attacking during a 10 minute exposure to one another, and the mean attack latency of 45 aggressive episodes per treated group of 10 mice. Although the data were not presented, the authors stated that no significant difference in the weights of the young at sacrifice were observed, and there was no overt evidence of illness or behavioral anomalies. The percent attackers were 100% and 96% in the two control groups. The percent attackers in the treated groups were 100%, 62%, and 90.5% in the low, medium and high exposure groups, respectively. The mean latent period for attack behavior in paired encounters was 4-10 times greater in the exposed mice. Latency was 27 ± 6.1 seconds and 43 ± 10.3 seconds in the two control groups. In the treated groups, latency was 27 ± 7.0, 320 ± 21.3, and 111 ± 16.5 seconds in the low, medium, and high exposure categories, respectively. A dose level of 0.01 ug/liter produced no change in behavior while aggression was depressed in the 0.1 and 1.0 ug/liter groups. Brain levels of serotonin, DOPA, and the biogenic amines were reported to be unchanged.

The acquisition of conditioned avoidance responses was delayed in the offspring of female mice exposed to DDT at 2.5 mg/kg in the second or third week of pregnancy (Al-Hachim and Fink, 1968). Another effect on behavior in offspring was demonstrated by Craig and Ogilvie (1974) who exposed female mice to technical DDT at 200 ppm in the diet throughout pregnancy and lactation. The offspring made significantly more errors and took significantly longer than controls when tested in a T-maze.

Tarjan and Kemeny (1969) conducted a six generation study using BALB/c mice fed 2.8 - 3.0 ppm p,p'-DDT in the diet. Groups of 28-30 parental animals were put on the treated diets six months prior to breeding. The investigators did not observe adverse effects on the number of pregnancies, number of births, number of litters, number of offspring or surviving weanlings, average body weight at weaning or average lifespan per generation. Tests of chemical impact on pup motility were also negative. Data were not reported by litter. The percent survival of pups to weaning was low (less than 65%) in the F0 and F1 generations for both control and dosed animals and remained low in the controls until the F4 and F5 generations. Therefore postnatal effects indicated by this parameter cannot be evaluated.

The p,p'-DDT isomer was also used in two studies designed to evaluate *in utero* toxicity. Lundberg and Kihlstrom (1973) mated NMRI mice, aged 40-60 days, and then administered p,p'-DDT by intraperitoneal injection on gestation day one, or on both days one and three of gestation. Dose levels provided were 0, 20, 50 and 100 mg/kg body weight. Dams, 18 - 35 per group, were killed on gestation days 10 - 15 and evaluated for the number of corpora lutea, placentae and embryos. Implantation was defined as the presence of placentae with or without an embryo. DDT administered on gestation day 1 only, caused a significant decrease in the number of implanted ova in all dams tested as well as in those dams possessing implantation sites. In the latter group, 88% of ova (indicated by number of corpora lutea) had implanted in control dams with implantation sites in contrast to 77% of ova implanted in females in the 50 mg/kg group. Ninety percent of ova had implanted in control females, while only 68% of ova had implanted in females treated with 100 mg/kg.

The 20 mg/kg dose level did not cause a decrease in ova implantation when administered on gestation day 1. A significant reduction did occur when this dose level was given on gestation days one and three. Only 75% of ova were implanted in treated females possessing implantation sites, whereas 88% of ova in control females in this category were implanted. No differences in the fetal developmental stage were noted at 15 days. It was suggested that this effect was due to an alteration of the hormonal balance possibly caused by increased enzyme induction and a resulting decrease in estrogen levels below the threshold necessary for successful implantation.

McLachlan and Dixon (1972), in a Society of Toxicology abstract, reported on an investigation of the effect of *in utero* p,p'-DDT exposure on the reproductive capacity of the offspring. Pregnant Swiss-Webster mice were exposed to 1 mg/kg p,p'-DDT on gestation days 10, 12, and 17. No gross teratogenic effects were observed, however the cellular components of the gonads were significantly altered in offspring. Fertility was also decreased in offspring mated. These effects were more marked in the females.

Dogs

Deichmann et al. (1971) observed subnormal reproduction in beagle dogs fed 12 mg/kg body weight p,p'-DDT by capsule five days a week for 14 months. Treated females were mated with treated males at the initial estrus after discontinuation of treatment. All of the 18 control dogs conceived and carried their litters to term. Sixteen delivered live pups, 84% of which survived to weaning. Four females were fed 12 mg/kg p,p'-DDT. Three were bred to males fed 6 mg/kg p,p'-DDT plus 0.15 mg/kg aldrin. Two females were bred two and three weeks after the treatment stopped and did not become pregnant even after 2 and 3 copulations. A third female delivered six pups, however four died within the first week. The other two survived to weaning. Mammary development and function was severely suppressed in the mother. The fourth female never came into heat during 12 months following discontinuation of treatment. One DDT treated male demonstrated a decreased libido. The dogs were reported to exhibit no obvious signs of intoxication. An increase in alkaline phosphatase activity was the only clinical abnormality noted in the DDT treated animals. Although a small number of DDT treated animals were evaluated, some quite overt reproductive effects were observed.

Beagle dogs responded minimally when exposed to 0, 1, 5, and 10 mg/kg body weight/day technical DDT "administered by mouth" from weaning through the remainder of their lives in the experiment (Ottoboni et al., 1977). Groups of 7-10 (F0), 12-19 (F1), and 30-44 (F2) dogs in each dose group were bred at puberty. A dose-related trend for earlier onset of puberty was significant in the F2 generation. The lack of significance in the other generations appeared to be affected by an unusual variation in the control animals. The authors concluded that the effect was DDT-related. No other differences were observed between treated and control animals in fertility index (percent of females bred that became pregnant), gestation index (percent of implantation sites that resulted in live pups), viability index (percent of live newborns that survived 24 hours), lactation index (percent of pups surviving 24 hours that were weaned), average litter size at birth and at weaning, and sex distribution at birth, in stillborn pups, and at weaning. In addition, no pathologic abnormalities attributable to DDT were found in brain, liver, kidneys, spleen, adrenals, gonads or thyroid. Finally, no congenital anomalies were attributable to exposure.

Conclusion and Weight of Evidence Classification

The lowest dose of technical grade DDT at which negative reproductive, prenatal or postnatal effects were reported in rats or mice was 2.5 ppm (0.125 mg/kg/day) described by Treon and Cleveland (1955). The incidence of preweaning mortality in Carworth rats was reported to be slightly increased in pups nursing dams exposed to 2.5 ppm (0.125 mg/kg/day). This dose level also significantly increased liver weights in females. Unfortunately, the data supporting these authors conclusions were not presented.

Decreased fertility and increased resorptions were caused in rats by 7 ppm DDT (0.35 mg/kg/day) (Green, 1969). Decreased fertility and postnatal mortality were caused by 250 ppm (32.5 mg/kg/day) DDT in Swiss White mice, a dose level which also caused maternal deaths (Keplinger et al., 1968). Survival was reduced in pups in the 100 ppm (13 mg/kg/day) group from birth to 4 days, 4 days to weaning, and from weaning to 120 days. The lowest dose level, 25 ppm (3.25 mg/kg/day), reduced survival to weaning in three of the six generations and in combination with low doses of aldrin or chlordane caused a wider range of effects.

The male offspring of mice exposed to 0.1 or 1.0 ug/liter technical DDT in their drinking water showed a significant decrease in aggressiveness (Scudder and Richardson, 1970). The mean latent period for attack behavior in paired encounters was 4 to 10 times greater in the exposed groups. The dose of 0.1 ug/liter is equivalent to 0.0002 mg/kg/day assuming a body weight of 0.03 kg and water consumption of 0.005 liters per day. A dose level of 0.01 ug/liter (0.00002 mg/kg/day) produced no change in behavior. Thus, behavioral anomalies were produced in the offspring of mice exposed to a very low dose rate of DDT. The significance of these effects is difficult to determine. A dose-response trend was not observed, and the mice exposed to the lowest dose showed the greatest level of aggression. Brain levels of serotonin, DOPA, and the biogenic amines were not changed.

Ovary weights were increased in female rats exposed to 2.5 ppm (0.125 mg/kg/day) o,p'-DDT but not in females exposed to 1.0 ppm (Wrenn et al., 1970). These investigators found no evidence of toxicity in the litters, however the number of animals per dose group was very small. A concentration of 1000 ppm (50 mg/kg/day) o,p'-DDT fed to female rats decreased pre-weaning growth in nursing pups (Clement and Okey, 1974). These F1 offspring also experienced decreased fertility, smaller litter size, and sterility due to ovarian cysts. It cannot be concluded that 20 ppm and 200 ppm o,p'-DDT have no biological effect because the number of females bred in each dose category was extremely small and less overt effects would not have been detected. Therefore, a no adverse effect level cannot be defined for o,p'-DDT in rats.

Pups nursing female Wistar rats fed 200 ppm p,p'-DDT experienced severely depressed growth but those that survived to weaning and were removed from exposure recovered (Clement and Okey, 1974). Fertility and litter size were not adversely affected. A dose level of 20 ppm also caused no ill reproductive effects. It cannot be concluded that these dose levels are truly not toxic because of the extremely small sample sizes used. Histologic alterations in gonads and decreased fertility were reported in male and female Swiss-Webster mice exposed to 1 mg/kg body weight p,p'-DDT on gestation days 10, 12, and 17 (McLachlan and Dixon, 1972).

A large number of studies of the reproductive and developmental toxicity of DDT have been conducted in mice and rats. Although several studies in rats have produced adverse effects, particularly at the higher dose levels used, only one report of reproductive toxicity in rats statistically evaluated the data. Resorptions were significantly increased in Sprague-Dawley rats exposed to 7 ppm DDT in the diet for 60 days before mating and for the duration of pregnancy (Green, 1969). One of the several studies conducted in mice reported a statistical evaluation of the results. Preweaning mortality was statistically increased in treated mice (100 ppm) compared to controls (Del Pup et al., 1978). Implantation frequency was significantly decreased in pregnant female mice when dose levels of p,p'-DDT as low as 20 mg/kg body weight were injected i.p. on gestation days one and three (Lundberg and Kihlstrom, 1973). The administration of 12 mg/kg body weight p,p'-DDT caused decreased fertility in beagle dogs which was quite extreme in the absence of signs of toxicity (Deichmann et al., 1971).

The production of increased resorptions in rats (Green, 1969), the increased preweaning mortality in mice (Del Pup et al., 1978) plus the epidemiologic evidence of developmental effects in the infants of North Carolina mothers associated with DDE levels in breast milk (Rogan and Gladen, 1982; Rogan et al., 1986) are sufficient to place DDT and its metabolites in the weight of evidence classification, probable positive for developmental effects. The two studies demonstrating the effect of p,p'-DDT on implantation frequency in mice

(Lundberg and Kihlstrom, 1973), and decreased fertility in beagles (Deichmann et al., 1971) are sufficient to place DDT in the weight of evidence classification, probable positive for reproductive effects, level II, animal data only.

The data on reproductive toxicity and developmental toxicity currently available in the literature are not sufficient to determine a NOAEL or LOAEL for use in developing a reference dose for human exposure to DDT. The literature contains the following deficiencies.

1. Conclusions were stated but the supporting data were not presented.
2. Information was not provided concerning various aspects of study design.
3. Sample sizes were too small to detect toxic effects at lower dose levels.
4. Control diets were not routinely monitored for contamination, or in one instance, were found to be contaminated.
5. Some authors do not present results in a way that allows for the impact of litter effect on the health of offspring.

THE CARCINOGENIC POTENTIAL OF DDT, DDE, AND DDD

EVIDENCE OF CARCINOGENICITY IN HUMANS

Several studies have evaluated the association of various diseases with different indexes of exposure to DDT and have suffered from the analytical problems characteristic of cross-sectional studies and case-control studies. A few cohort studies have also been conducted, primarily involving pesticide applicators and DDT manufacturing workers. Although these studies are limited and are difficult to interpret, a possible association of DDT exposure with respiratory cancer is suggested.

Cross-Sectional Studies

Some researchers have attempted to establish an association between tissue concentrations in autopsy samples and the disease state of the individual. The results have been suggestive of a possible connection, however no causal association can be inferred because the time of exposure in relation to the onset of disease cannot be established in cross-sectional studies.

Radomski et al. (1968) determined organochlorine pesticide concentrations in fat and liver autopsy specimens of 271 patients previously exhibiting various pathological states of the liver, brain and other tissues of the body. All autopsies with a history of liver, brain or neurological disease were selected between January 1964 to June 1967. These were to be compared with a randomly selected group with normal livers and brains. The next of kin were questioned regarding demographic characteristics and various factors which may have had an impact on the tissue concentrations measured. Those individuals with portal cirrhosis of the liver were found to have mean fat levels of p,p'-DDE and dieldrin that were significantly higher than those of the normal population ($0.01>p>0.001$ and $P<0.001$, respectively). The pesticide levels in fat and liver tissue were not different between individuals with fatty metamorphosis of the liver and those with normal livers. Metastatic malignancy of the liver in 30 cases was associated with p,p'-DDE concentrations in fat three times as high as normal ($p<0.005$) and seven times as high as normal in the liver tissue ($p<0.001$). The concentrations of p,p'-DDT were also elevated in the fat, but not the liver. Levels of dieldrin and p,p'-DDD were not different from normal. The authors believed that a consistent increase in all pesticide levels would have been expected if the association was due to an increase in fat content of the liver associated with disease. The 24 cases of encephalomalacia had significantly higher levels of p,p'-DDE in fat ($0.01 > p > 0.001$) and the brain ($0.05 > p > 0.01$) when compared to the normal sample. Consistent elevated concentrations ranging from two to four times the normal of p,p'-DDE, dieldrin, p,p'-DDD and p,p'-DDT were reported in eight cases of essential hypertension. Finally, pesticide concentrations averaged two or three times the normal concentration in the fat of 40 cases of various types of carcinoma. A relationship was found between concentration in fat and exposure from residential pesticide use when the cases were classified according to their relative degree of home pesticide use.

This study has several interpretive problems. Individuals selected into the study were reclassified after the tissue samples were evaluated histologically and medical records were reviewed. For example, cases thought to have liver disease before autopsy were found to have only chronic passive congestion secondary to myocardial infarction, and cases in the normal group were found to have diseases such as hypertension and malignancies. The authors decided to evaluate the identified diseases for their association with tissue concentration. Therefore, the definition of the normal comparison group became unclear. The need to adjust tissue levels in compared groups for differences in age composition or by sex was not discussed by the authors. In addition, the authors recognized that the elevated pesticide concentrations could have been caused by the disease state or vice versa.

The association of PCB and DDE levels in the adipose tissue of deceased persons with and without cancer in Denmark was discussed in two reports by Unger et al. (1980 and 1982). Subcutaneous abdominal adipose tissue samples from 71 terminal patients in Denmark were analyzed in 1978 and 1979. Twenty-seven of the subjects had died of cancer (11 men and 16 women) and 44 died of other causes (22 men and 22 women). The mean DDE and PCB values for all samples were 3.55 ppm and 6.55 ppm in extractable lipid, respectively. A significant age correlation was established by regression analysis for DDE levels in the cancer ($p<0.03$) and noncancer groups ($p<0.0046$). PCB and DDE concentrations were also significantly correlated with disease status (cancer and noncancer) when the data were adjusted for age and sex ($p<0.01$ and $p<0.001$, respectively).

A second study presented the results of a statistical reanalysis of the data to account for several other potentially confounding variables which might influence the relationship between cancer and the tissue concentration of the two organochlorines. The sample had been expanded to include adipose tissue from 51 cancer patients and 63 persons who neither suffered from nor died of cancer. All cancers were histologically verified by biopsy and/or autopsy. PCB and/or DDE were statistically associated with cancer mortality after controlling for all potential confounders including a height-weight index, age, sex, farmer/nonfarmer, and urban/rural residence. Exposure to other potential carcinogens or mixtures and smoking habits were not evaluated. These authors also acknowledged that it is impossible to determine whether the high concentrations were etiologically related to the development of cancer or were merely a result of it.

Unger and his associates subsequently investigated the relation of PCB and DDE in breast fat tissue to the existence of mammary cancer in recently diagnosed patients (Unger et al., 1984). The investigators analyzed mammary human female adipose tissue taken at autopsy from deceased women with breast cancer and without breast cancer. Breast fat tissue was also analyzed from living patients with breast cancer and age-matched (+or- 5 years) with those with other disorders of the breast all undergoing breast surgery. PCB concentrations were a mean of 6.47 ppm in the autopsy specimens of breast cancer patients and 5.12 ppm in non-breast cancer patients, however the difference was not significant when controlling for age in a linear logistic regression model. DDE concentrations were not compared in the autopsy samples. PCB and DDE content in mammary tissue was also not significantly different between breast cancer cases and other breast disease cases when evaluated controlling for age, percent fat, and DDE or PCB. Differences in breast-feeding behavior between the two groups were not evaluated. Breast-feeding behavior is a potential confounder because it has been associated with both organochlorine concentration in fat and perhaps with the disease state. The authors concluded that human mammary tissue is not a target organ for PCB nor DDE concerning carcinogenesis.

A study of serum levels of a small number of individuals with colorectal carcinoma, aged 14 to 19 years, in Arkansas, Mississippi, and Tennessee did not find significantly higher serum DDT levels than in a control sample. The control contained residents of northwestern Mississippi, 5 to 18 years of age, attending a health clinic (Caldwell et al., 1981). Mean serum levels for total DDT were 65.56 ppb with a range of 5 to 230 ppb in cases, while levels in controls were 28.25 ppb with a range of 7 to 96 ppb.

Case-Control Studies

Several case-control studies of soft tissue sarcomas (STS) and exposure to chlorophenols and phenoxyacetic acids have also evaluated exposure to DDT. Hardell and Sandstrom (1979) calculated an odds ratio of 1.2 from four out of

52 soft-tissue sarcoma cases and 14 out of 208 matched controls who reported exposure to DDT. A study of 169 malignant lymphoma cases and 335 controls matched for age, sex and place of residence found that DDT exposure was more frequent in the cases than the controls (13% vs 7.8%) (Hardell et al., 1981). The percent of DDT exposed individuals was also higher among cases when those exposed to phenoxy acids were excluded (5.5% vs 3.5%). The authors noted that DDT exposure co-varied with exposure to phenoxy acids. This may have caused a positive bias. The odds ratio calculated for phenoxy acid exposure was 4.8.

Another study of soft-tissue sarcoma conducted in southern Sweden found that 6.4 percent of 110 cases of soft-tissue sarcoma reported exposure to DDT compared to 5.0 percent of the 219 controls matched for age and residence (Eriksson et al., 1981). This risk ratio of 1.3 was not statistically significant. The risk ratio for phenoxy acids was 6.8.

Everett et al. (1984) reported the results of a population-based case-control study in Iowa and Minnesota evaluating links between farming and non-Hodgkin's lymphoma (NHL). An elevated risk was indicated for several pesticides including DDT (Odds Ratio = 1.5, $p<0.05$) in a total of 622 cases and 1245 matched controls. A recent study of soft tissue sarcoma and non-Hodgkin's lymphoma in relation to phenoxyherbicide and chlorinated phenol exposure in Western Washington reported a pooled odds ratio of developing non-Hodgkin's lymphoma significantly elevated over controls matched to NHL cases by vital status and five-year age group (1.82, 95% CI 1.04-3.2) (Woods et al., 1987). However, DDT exposed individuals represented only 4 percent of the study population of 576 men with NHL diagnosed between 1981-1984 and 694 population controls. The pooled odds ratio for DDT exposure for 128 men with soft tissue sarcoma (STS) compared to the controls was 1.1 (95% CI 0.4-3.2). Phenoxy herbicide exposure was not significantly associated with STS or NHL in this study.

Although useful for generating hypotheses, the studies of STS and NHL have limited value for determining the carcinogenic potential of DDT alone. These studies all involved multiple exposures, and it is impossible to determine the carcinogenic potential of DDT alone.

Excesses of leukemia have been indicated in relation to DDT exposure. An abstract by the researchers of the Iowa/Minnesota study population reported an association between DDT use and leukemia among 578 pathologically confirmed cases and 1245 controls frequency-matched by year of birth and vital status (Blair et al., 1984). An odds ratio of 1.4 was reported without discussion of its statistical significance.

A case-referent study was reported by Flodin et al. (1988) which investigated differences in exposures in patients with chronic lymphatic leukemia compared to selected controls. The cases were obtained from the registers of cytological departments at three Swedish hospitals and the medical clinics at two hospitals. Diagnoses were confirmed by cytologists and clinical physicians. Criteria for inclusion included age below 80 years, mentally capable of answering a questionairre, Swedish born, and resident in the catchment areas of the particular hospitals at the time of diagnosis. A total of 111 cases agreed to participate. Referents (431) were randomly drawn from population registers corresponding to the catchment areas of the appropriate hospital. The age of referents was restricted to between 40 and 80 years, the age range of the cases. Exposure was assessed by questionairre and a minimum of one year of exposure was required. The last five years of exposure were disregarded to allow for a five year latency.

Several exposure categories were found to be significantly associated with leukemic lymphoma using multiple linear regression. These included age greater than 60, engine exhaust, DDT, and working in contact with fresh wood (paper pulp industry, lumberjacks, sawmill workers). DDT was also significantly associated with lymphoma along with age greater than 60, engine exhausts, and contact with horses when Mantel-Hentzel rate ratios were calculated. The rate ratio was relatively large (6.0, 95% conf. limits: 1.5 - 23) but only four out of 111 cases and six out of 431 referents were exposed to DDT. Moreover, no information was available concerning the degree of exposure. In addition, the two populations may have exhibited a differential recall of exposure.

DDT exposure was not related to the presence of colon cancer among male patients, aged 25-85, in Sweden compared to controls from the same population stratified for age and place of residence (Hardell, 1981). Exposure to phenoxy acids or chlorophenols was also not elevated in colon cancer patients.

Cohort Studies

The World Health Organization organized two studies to investigate the long-term effects of exposure to DDT. In Brazil, 202 spraymen exposed to DDT for six or more years, 77 workers exposed for 13 years, 78 men who lived in houses sprayed indoors with DDT every six months, and 406 controls were given periodic clinical examinations. Minor differences in neurological tests were noted between the control and exposed population at the first clinical examination, however these results were not confirmed in subsequent examinations. Significant increases in DDT and DDE residues in the blood serum levels were observed in the exposed population.

A survey of 144 spraymen and 125 controls in India showed similar results. Minor neurological problems such as brisk knee reflexes, slight tremors, and poor performance in a timed Romberg test were observed at the first clinical examination. These findings were not confirmed in 20 men re-evaluated by a neurologist.

The two WHO studies did not evaluate mortality and 13 years may not have been long enough to allow the development of cancer. Therefore, no conclusions can be drawn from these studies regarding the carcinogenicity of DDT in humans exposed for long periods.

Two retrospective cohort mortality studies conducted in the United States did not find a definitive association with cancer mortality and DDT exposure. These studies possibly lacked the statistical power to detect an increased incidence of cancer.

During the 1970's, a retrospective cohort study of workers in four organochlorine manufacturing plants was initiated by the National Institute for Occupational Safety and Health (NIOSH) (Ditraglia et al., 1981). One of the four plants had produced DDT exclusively since 1947. Standardized mortality ratios (SMR) were calculated using a modified life table analysis program and corresponding U.S. white male cause-specific mortality rates. Of the 42 workers known to be deceased in the cohort of 354 workers (7601 person-years) at the DDT plant, no excess for total mortality, all malignant neoplasms, or other causes of death were found. Cause specific mortality was also not significantly increased for any specific cancer types. A slightly increased SMR for cancer of the respiratory system was associated with four of the six observed cancer cases (SMR=125). When the deaths from malignant neoplasms were examined by latency (< 10 years, 10-19 years, > 20 years), an increase in risk was noted with an increase in the length of the latency period. Five of the individuals with malignant neoplasms had worked for 20 or more years, and the one additional worker had been employed at the plant between 10 to 19 years.

The authors acknowledged that due to the small number of workers included in this study the statistical power did not enable them to conclude that no association exists between cause-specific mortality and employment at the study plants. Rapid turnover of workers is characteristic of this industry, and approximately 70% of the employees at the DDT plant worked less than six months. Other factors in the study design may have led to an underestimate of risk. Vital status was unknown for 10% of the cohort. Therefore complete ascertainment of mortality may not have been accomplished. In addition, this 10% was assumed to be alive at the cut-off date for including deaths which may have given an overestimation of the actual number of person-years at risk producing an inflated number of expected deaths. In addition, only workers who had been employed for more than six months were included in the cohort perhaps excluding those who may have later developed cancer. Some of the workers had been exposed for a maximum of 12 years. This may not have allowed for a long enough latency period leading to a potential negative bias.

Another cohort of chemical workers employed between 1935 and 1976 at three manufacturing plants in Michigan and Arkansas, and a Michigan research establishment with potential exposure to brominated chemicals was studied

(Wong et al., 1984). Of the 3579 employees in the cohort, 578 (16.2%) had died by December 31, 1976, the termination date for follow-up. Vital status was not determined for 195 (5.4%) workers. SMRs were calculated in a manner similar to that used in the Ditraglia et al. study using the U.S. white male age-cause specific mortality rates for five year periods from 1935 to 1975.

Of the total cohort, 740 individuals had worked in DDT production departments. An excess in respiratory cancer was observed in this group. Ten cases were observed when 6.43 were expected and the SMR was 156. The increase was not statistically significant. No other increases in cause-specific cancer incidence were observed in this group. The authors pointed out that these workers were also potentially exposed to other chemicals, including inorganic brominated compounds. Inorganic brominated compounds were associated with a significant increase in respiratory cancer (SMR=308, $p<0.05$). An underestimation of risk in this study was less likely than in the Ditraglia et al. study because workers for which vital status was unknown were considered lost to follow-up and their contribution to person-years followed was terminated on the date employment ended. This percentage was also relatively low. Workers were classified into exposure categories on the basis of work history. Therefore, misclassification bias is a possibility. Previous employment and smoking habits were not evaluated which is important, particularly for respiratory cancer.

Both cohort studies indicated possible problems with malignancy associated with DDT, specifically respiratory cancer. However, slight increases observed were not statistically significant, and the workers were exposed to other potentially carcinogenic chemicals. Both authors emphasized that a longer observation period was needed to establish any trends in mortality that exist.

Other cohort studies in agricultural workers (Barthel, 1981) and pesticide applicators (Wang and MacMahon, 1979; Blair et al., 1983) exposed to a variety of pesticides and herbicides found increased mortality from lung cancer compared to the national and regional mortality rates. Barthel (1981) studied a group of 1658 male workers who had been active for at least five years as agricultural plant protection workers or plant protection agronomists in 14 districts of the German Democratic Republic during 1948 to 1972. A total of 50 bronchial carcinoma cases were observed in agricultural workers between 1970 to 1978, 1.8 times the 27.5 cases expected. The excess cancer incidence was statistically significant ($p<0.05$). The SMR increased with the duration of employment from 1.2 to 1.7 to 3.0 in workers exposed for less than 10 years, 10 to 19 years, and more than 19 years, respectively.

The employees of three nationwide pest control companies exposed between January 1, 1967, or January 1, 1968 and June 30, 1976 were evaluated in relation to pesticide exposure and mortality (Wang and MacMahon, 1979). A slight increase in lung cancer was observed (SMR=115) which was not statistically significant (24.3 cases). The SMR for skin and bladder cancer were also elevated, but the number of cases was very small. Only about 15% of the person-years of follow-up were contributed by workers who had more than five years of employment and who were observed more than 10 years after first employment.

An elevated mortality from lung cancer was also reported for a cohort of pesticide applicators in Florida (Blair et al., 1983). Vital status as of January 1, 1977 was ascertained for 4411 pest control applicators licensed during 1965-1966. Although the minimum period of follow-up was only ten years, the SMR for lung cancer was 135 using U.S. age-specific mortality rates. The risk increased with the number of years licensed and was 101, 155, and 289 for those licensed for less than 10 years, for 10 to 19 years, and for 20 years or longer, respectively. The use of 1970 to 1975 Florida mortality rates did not alter the calculated SMRs.

No conclusions regarding the carcinogenicity of DDT can be drawn from the studies of pesticide applicators. Exposure to multiple chemicals were involved, and no evaluations of smoking habits were conducted. However, the consistent finding of an elevated lung cancer mortality in these studies and those of DDT manufacturing workers should be noted with concern.

EVIDENCE OF CARCINOGENICITY IN EXPERIMENTAL ANIMALS

The carcinogenic potential of DDT, DDD, and DDE has been studied extensively in experimental animals. The citations and study designs for all species evaluated are presented in Table 11. Tumor incidence has not been consistent across species tested in long-term bioassays.

Out of 10 chronic studies of DDT exposure conducted in mice, 8 were positive for carcinogenicity in either or both sexes. Although liver was the dominant site for cancer in mice (5 studies), an increased incidence of lung adenoma and carcinoma (3 studies), and lymphoma (3 studies) were also observed. Two studies evaluated the carcinogenicity of DDE in mice and were positive for liver cell tumors in both strains. DDD was associated with an increased incidence of lung adenoma in both sexes in one study.

Ten chronic studies of DDT exposure were conducted in rats of which three were positive for liver tumors in either or both sexes. One study which evaluated the carcinogenicity of DDD and DDE in rats did not observe liver tumors, Treatment with DDD was associated with thyroid tumors in males.

Four bioassays in hamsters exposed to DDT were negative for carcinogenicity. One study of DDE exposure in hamsters was positive in both males and females. Bioassays in dogs and monkeys treated with DDT have also been negative for cancer.

The carcinogenicity bioassays are not all of good quality, and the results of the studies should not all be given equal weight. The studies are summarized in this section with a discussion of their relative quality for the purpose of establishing the weight of evidence for the carcinogenic potential of DDT, DDD and DDE in the species tested.

Table 11. Studies of Carcinogenicity in Experimental Animals exposed to DDT, DDD, and DDE.

Species/ Sex/ Reference	DDT	Dose (ppm)	Dose Rate (mg/kg/day)	#/ sex/ group	# at Risk	Duration of Study (Weeks)
MICE						
C3H/AnF x C57Bl/6 AKR x C57Bl/6 Both sexes Innes et al., 1969	p,p'-DDT	0	0	90	?	80
		140	21	18		
BALB/c Both sexes Tarjan et al., 1969	p,p'-DDT	0		406	?	6 gen.
		2.8-3	0.4-0.7	683		113
CF1 Both sexes Walker et al., 1972	p,p'-DDT	0	6.5	47	?	112
		50	13	32		
		100		32		

Table 11 continued. Studies of Carcinogenicity in Experimental Animals exposed to DDT, DDD, and DDE.

Species/ Sex/ Reference	DDT	Dose (ppm)	Dose Rate (mg/kg/day)	#/ sex/ group	# at Risk	Duration of Study (Weeks)
CF1	p,p'-	0	0	45	M/F 45/44	110
Both sexes	DDT	100	13	30	M/F 29/27	
Thorpe &						
Walker, 1973						
A strain		0	0	?	206	5 gen.
Both sexes		10	1.5		234	45
Shabad et al.,		50	7.5		30	
1973						
BALB/c	Technical	0	0	M/F 107/131		2 gen.
Both sexes		2	0.3	M/F 112/135		140
Terracini		20	3.0	M/F 105/128		
et al., 1973		250	37.5	M/F 106/121		
CF1	Technical	0	0		M/F 328/340	6 gen.
Both sexes		2	0.3		M/F 354/339	P: 132
Turusov et al.,		10	1.5		M/F 362/355	F: 130
1973		50	7.5		M/F 383/328	
		250	37.5		M/F 350/293	
Swiss	Technical	0	0	30	M/F 26/20	80
Both sexes		100	13		M/F 27/22	
Kashyap et al.,			10		M/F 24/24	
1978						
B6C3F1 M/F	Technical	0	0	20		93
Both sexes M		22	3.3	50		
NCI, 1978 M		44	6.6	50		
F		87	13.1	50		
F		175	26.25	50		
CF1	p,p'-DDE	0	0	M/F 100/90	98/90	123
Both sexes		250	32.5	60	53/55	
Tomatis et al.,						
1974						
B6C3F1	DDE	0	0	20	M/F 8/19	93
Both sexes		148	22.2	50	M/F 38/47	
NCI, 1978		261	39.2	50	M/F 36/48	
CF1	p,p'-DDD	0	0	M/F 100/90	98/90	123
Both sexes		250	32.5	60	59/59	
Tomatis et al.,						
1974						

Table 11 continued. Studies of Carcinogenicity in Experimental Animals exposed to DDT, DDD, and DDE.

Species/ Sex/ Reference	DDT	Dose (ppm)	Dose Rate (mg/kg/day)	#/ sex/ group	# at Risk	Duration of Study (Weeks)
B6C3F1	DDD	0	0	20		93
Both sexes		411	53.4	50		
NCI, 1978		822	106.9	50		
RATS						
Osborne-Mendel	Technical	0	0	12	?	104
		200	10			
Both sexes		400	20			
Fitzhugh &		600	40			
Nelson, 1947		800	80			
Wistar	Technical	0	0	34-36	M/F 35/32	145
Both sexes		500	35		M/F 27/28	
Rossi et al., 1977						
Wistar	Technical	0	0	30-38	?	144
Both sexes		125	6			
Cabral et al.,		250	12			
1982		500	24			
Albino	Technical	0	0	?	?	60
Both sexes		3.5	0.36			
Cameron &		35	3.6			
Cheng, 1951		350	36			
Carworth	Technical	0	0	40	?	104
Both sexes		2.5	0.1			
Treon &		12.5	0.5			
Cleveland,		25	1			
Osborne-Mendel	Technical	0	0	30	?	104
		80	3.2			
Both sexes Ramdoski et al., 1965						
Osborne-Mendel	Technical	0	0	30	?	116
		200	8			
Both sexes Deichmann et al., 1967						

Table 11 continued. Studies of Carcinogenicity in Experimental Animals exposed to DDT, DDD, and DDE.

Species/ Sex/ Reference	DDT	Dose (ppm)	Dose Rate (mg/kg/day)	#/ sex/ group	# at Risk	Duration of Study (Weeks)
Fisher Both sexes Weisburger & Weisburger, 1968	Technical		0	15	?	78
			15			
Osborne-Mendel Both sexes NCI, 1978 1955	Technical		0	20	?	111
			M/F 16.1/10.5	50		112
			M/F 32.1/21.0	50		113
	DDD		0	20	?	111
			M/F 82.4/42.5	50		112
			M/F 164.7/85.0	50		113
	DDE		0	20	?	111
			M/F 21.9/12.1	50		111
			M/F 42.0/23.1	50		111
HAMSTERS						
Golden Syrian Both sexes Agathe et al., 1970	Technical	0	0	30	?	48
		500	48			
		1000	96			
Golden Syrian Both sexes Graillot et al., 1975	Technical	0	0	30	?	78
		250	24			
		500	48			
		1000	96			
Golden Syrian Both sexes Cabral et al., 1982	Technical	0	0	40	M/F 39/40	104
		125	12	30	M/F 28/30	
		250	24	30	M/F 28/31	
		500	48	40	M/G 40/39	
Golden Syrian Both sexes Rossi et al., 1983		0	0	45/46	M/F 31/42	104
	Technical	1000	96	48	M/F 35/36	
	DDE	500	48	47/45	M/F 30/39	
	DDE	1000	96	40/43	M/F 39/39	

An early experiment evaluated the carcinogenicity of 123 pesticides and industrial compounds in mice. Five pesticides, including p,p'-DDT, and 6 other chemicals significantly increased tumor incidence in two hybrid mice strains tested (Innes et al., 1969). The hybrids were bred by crossing C57Bl/6 with either C3H/Amf or AKR strains and the F1 generations were designated X and Y respectively. The maximum tolerated dose, 46.4 mg/kg in a 0.5% gelatin suspension, was given by gavage to the mice (18 males and 18 females) from 7 days of age to 28 days of age. The chemical was then administered in the diet at a rate of 21 mg/kg/day (140 ppm) from 4 weeks of age to 81 weeks of age at which time the animals were killed. A control group of 90 males and 90 females was also followed.

Treatment had no impact on the survival of males. In females, 61% (11/18) of the treated X strain mice survived to 81 weeks compared to 92% (83/90) of the control X strain mice. Survival in the treated Y strain mice was 72% (13/18) compared to 83% (75/90) in the controls. Body weight gain was not reported.

A significantly increased incidence of hepatoma was observed in both sexes and strains compared to controls, and the incidence of malignant lymphomas was significantly increased in Y strain females compared to control females. Tumor incidence is presented in Table 12. A corrected relative risk was calculated to compare tumor incidence among treated mice with incidence among controls. The relative risk for hepatoma was significantly elevated for males of both strains, and for X strain females ($p<0.01$). The elevated relative risk was also statistically significant for both sexes when the data for both strains were combined, and for both strains when the data for males and females were combined. The relative risk for lymphoma was elevated in both sexes and strains, but was significant only in Y strain females, in females when data on both strains were combined, and in the Y strain when data on males and females were combined ($p<0.01$). The relative risk for pulmonary tumors was also elevated, but the increase was not statistically significant.

The hepatomas did not metastasize, however the authors stressed that they were not necessarily benign. The pulmonary tumors were primarily adenomas, and most of the lymphomas were identified as Type B reticulum-cell sarcomas.

Table 12. Tumor Incidence in Two Strains of Mice Fed 46.4 mg/kg p,p'-DDT by Gavage for 4 Weeks Followed By An Additional 76 Weeks of 140 ppm p,p'-DDT in the Diet (Innes et al., 1969).

	Liver Tumors				Lymphoma			
	X Strain		Y Strain		X Strain		Y Strain	
Dose ppm	Male	Female	Male	Female	Male	Female	Male	Female
0	8/79[1] (10.1%)	0/87 (0%)	5/90 (5.6%)	1/82 (1.2%)	5/79 (6.3%)	4/87 (4.6%)	1/90 (1.1%)	4/82 (4.9%)
140	11/18* (61.1%)	4/18* (22.2%)	7/18* (38.9%)	1/18 (5.6%)	0/18 (0%)	1/18 (5.6%)	1/18 (5.6%)	6/18* (33.3%)

[1] Number of Tumors Compared to Number of Animals Necropsied.
* Statistically Significant, $p<0.01$.

The increased mortality among female mice may have interfered with tumor incidence. The authors did not adjust for intercurrent mortality when evaluating the data. This may account for the lack of significance for the relative risk of hepatoma in Y strain females and the lower incidence among females. The number of animals treated per sex was also quite low, decreasing the power of the test to detect significant increases in incidence. In addition, the animals were treated for only 80 weeks and were not followed for a lifetime. Even so, a significantly increased incidence of liver tumors and lymphoma was detected in the mice.

Another early study by Tarjan and Kemeny (1969) found increased numbers of leukemias, pulmonary carcinoma, and other tumors in mice followed over six generations. A diet of 2.8 - 3.0 ppm (0.4 - 0.7 mg/kg/day) p,p'-DDT or a control diet which contained 0.2 - 0.4 ppm p,p'-DDT was given to the mice during the multigeneration test. Mice were fed the test diet for six months and were then switched to the control diet. After 26 months, 683 treated mice and 406 control mice were selected from all surviving generations. Leukemia incidence (myeloid, lymphoid, and aleukemias) was significantly increased in the F3, F4, and F5 generations ($p<0.008$). A total of 85 out of the 683 animals (12.4%) were found to have leukemia compared to 10 of the 406 controls (2.5%), and incidence was described as particularly striking in females. Spontaneous leukemia was reported to be unknown in BALB/c mice. Pulmonary carcinoma was the predominant tumor type; 59% of the animals with tumors had pulmonary carcinoma. A total of 116 of the 683 treated mice (17%) had pulmonary carcinoma (significantly higher than controls, $p<0.007$). Background incidence of this tumor in this colony was reported to be below 0.1%. Other tumors including malignant vascular tumors and reticulosarcomas of the liver, kidney, spleen, ovary, and other organs were reported. The number of total tumor bearing animals was significantly higher in treated mice than controls (28.7 vs 3.2%), and the incidence of tumor bearing mice increased in succeeding generations.

This study demonstrated that DDT increases the incidence of tumors in mice, particularly lung adenomas and leukemia, at dose levels as low as 3 ppm in the diet. The study was not well reported which makes its evaluation difficult. No information was provided regarding survival or growth. Tumor incidence data were reported for treated mice and controls (males and females) for leukemia and total tumors only. Although the study was conducted for 26 months, it is not clear whether individual animals were followed for a lifetime. The mice were treated for only six months which would have limited application for predicting cancer incidence over a lifetime of exposure.

Two studies by Walker et al. (1972) and Thorpe and Walker (1973) were positive for tumorigenicity in the livers of CF1 mice. Dietary dose levels of 50 ppm p,p'-DDT (7.5 mg/kg/day) and 100 ppm p,p'-DDT (15 mg/kg/day) were given to CF1 strain mice for 112 weeks (Walker et al., 1972). A sample size of 32 mice per sex per dose level was used for treated animals, and 47 mice per sex were used in the control group. Animals were palpated to detect liver enlargements at various time periods, and individuals were killed when the enlargement was considered to be life threatening. No conclusions regarding the effect of DDT on survival can be made from the reported data. Morbidity was defined as the number of mice found dead, showing signs of ill-health and sent for autopsy, or sent for autopsy because of the size of an intra-abdominal mass. Morbidity was reported to be lower than controls in males treated with 100 ppm DDT compared to controls. Body weight gain was not reported.

Enlargements were detected in the livers of the 100 ppm group at 65 weeks and in the livers of the 50 ppm group at 96 weeks. All animals received a histopathological exam upon sacrifice, and an increase in liver cell tumors was observed in the treated mice compared to control mice. Tumor incidence is presented in Table 13.

Table 13. Liver Tumor Incidence in CF1 Mice Fed 50 and 100 ppm p,p′-DDT for 112 weeks (Walker et al., 1972).

Dose		Number of Animals[1]		Liver Tumors (Number/Percent)	
ppm	Tumor Type[2]	Male	Female	Male	Female
0	All	47	47	6 (13%)	8 (17%)
	Type a			6	8
	Type b			0	0
50	All	32	30	12 (37%)	15 (50%)
	Type a			9	13
	Type b			3	2
100	All	32	32	17 (53%)	24 (76%)
	Type a			14	20
	Type b			3	4

[1] Number of Animals Necropsied.

[2] Tumor Type: All = Type a + Type b; Type a = Hyperplastic nodules and benign hepatomas; Type b = Malignant tumors.

The data indicate that DDT has a dose-related effect on liver tumor incidence, and that females responded with a higher incidence than males. Statistical tests were not used to evaluate the data. Two types of tumor were classified in the livers. Type a lesions, labelled benign tumors, were comprised of hyperplastic nodules and benign hepatomas since "a clear-cut distinction was not possible." Type b lesions, stated to be uncommon in control mice, were distinguished from type a tumors by the presence of areas of papilliform and adenoid formation of liver cells with wide and irregular vascular channels within the growth. The majority of type b tumors were found in livers that also had type a lesions. One female mouse fed 100 ppm DDT showed type b liver tumors with metastases to the lungs. In conclusion, although DDT exposure clearly had a tumorigenic effect on the liver, the degree of response cannot be determined in relation to relative survival between groups because of the practice of killing the animals when palpated tumors were considered to be life threatening.

Thorpe and Walker (1973) fed the same high dose level, 15 mg/kg/day, to CF1 mice for 110 weeks. In contrast to the previous study, mice were not autopsied when the palpated abdominal masses reached a size considered to be injurious to health. Survival was reported to be similar in treated and control mice. Body weight gain was not reported. Liver enlargement was first observed at week 68 and at week 50 in treated males and females, respectively. The first liver tumors were observed at 78 weeks and at 100 weeks in control males and females, respectively.

Histopathological examinations resulted in the identification of a statistically significant increase in liver tumors in treated males and females. Liver tumors were classified in the same manner as in the previous study (Walker et al., 1972), and the pathological description of these tumors was identical. Tumor incidence in the liver is summarized in Table 14. When evaluated as the percent of animals that survived to the time that a tumor was identified in any tissue, 79% and 96% of the treated males and females had liver tumors compared to 24% and 23% of the control males and females. A lower tumor incidence was reported in the lung, lymph tissue, and kidney in treated mice.

Table 14. Liver Tumor Incidence in CFl Mice Fed 100 ppm p,p'-DDT Over a Lifetime (Thorpe and Walker, 1973).

Dose		Number of Animals[1]		Liver Tumors (Number/Percent)	
ppm		Male	Female	Male	Female
0	All	45	44	11 (24%)	10 (23%)
	Type a			9	10
	Type b			2	0
100	All	29	27	23 (79%)*	26 (96%)*
	Type a			14	14
	Type b			9	12

[1] Number of animals surviving to the time that a tumor was found in any tissue in each group of mice.

* Significantly different from control, $p<0.01$.

A multigenerational study was conducted by Shabad et al. (1973) in A-strain mice. Mice in the parental generation were dosed with 1.5 and 7.5 mg/kg/day technical DDT via gavage (in 0.1 ml sunflower oil), while the F1 - F5 generations were dosed with 1.5 mg/kg/day. All animals were dosed beginning at 6 to 8 weeks of age, and treatment was continued to 52 weeks of age. Almost 50% of the high dose parental mice died prior to six months. Reproduction was affected, and the females died before parturition with convulsions and paralysis. An effect on reproduction and general toxicity was also reported in the low dose group. Approximately 59% of the parental generation died before six months. Mortality in controls was not reported.

The control group had an overall incidence of lung adenoma of 7% while the parental generation receiving the highest dose had an incidence of 37%. The incidence of lung adenomas in the low dose parental mice and subsequent generations was 19%(F0), 15%(F1), 24%(F2), 46%(F3), 43%(F4), and 13%(F5). The authors stated that A strain mice are very susceptible to the development of lung tumors. The appearance of tumors was earlier in the F2-F5 generations than in the parental and F1 generations. Lung adenomas appeared in mice dying in the later generations prior to six months, whereas the lesions did not appear prior to six months in the parental, F1, or control generations. Tumors were not reported in other organs, although it was not clear whether or not other organs in addition to the lung were examined.

The study was not well reported making the interpretation of its results more difficult. Data were not reported by sex. In addition, statistical techniques and pathological methods were not described. Since the animals were followed for only 52 weeks, it is not surprising that liver tumors were not observed.

Terracini et al. (1973) conducted two studies using two colonies of BALB/c mice and three dose levels of technical DDT. The mice were treated with 0, 2 ppm (0.3 mg/kg/day), 20 ppm (3.0 mg/kg/day), and 250 ppm (37.5 mg/kg/day) DDT beginning at 4 to 5 weeks of age through the remainder of their lifespan (140 weeks). The diets contained 3% olive oil. The DDT concentration in the control diet was found to be 0.02 to 0.09 ppm. Dieldrin was measured twice at 0.01 and 0.05 ppm, and gamma-BHC was repeatedly found ranging between 0.01 and 0.3 ppm. No aflatoxins were measured. Survival of females in both generations was comparable between treated mice and controls, except the lifespan of F1 females was shortened after the 80th week. Mortality in male mice was much higher than controls in all three dose groups due to wounds and urinary infections produced by fighting. Convulsions also caused higher mortality in the 250 ppm group as early as 12 weeks of age. No differences in body growth or average weight were reported in the mice up to 80 weeks of age. Weight loss was increased in older males and females in the 250 ppm group.

The percent of tumor bearing animals varied between 76% and 90% in the controls and dose groups with no treatment-related effect. Liver cell tumors were significantly increased in the 250 ppm group for males and females in both generations ($p<0.01$). Tumor incidence is summarized in Table 15. Both colonies showed the same response. Therefore, the incidence data were pooled. The incidence of liver cell tumors in the 250 ppm females was 44% in the parental and 74% in the F1 generation compared to no hepatic tumors in the controls. The earliest liver tumors were found in an F1 mouse at 77 weeks. The incidence of liver cell tumors was higher in the F1 mice, and the tumors appeared earlier and in greater number. In males, 14% of the parental and F1 mice combined had liver cell tumors compared to 2% of the control mice. Liver cell tumors were observed in male mice dying before 59 weeks of age. The incidence of lymphoma was similar in the control and two lower dose groups (50%) and was decreased in the highest dose group to 14% in one colony and 36% in the other. The incidence of lung adenomas was not significantly different between treated and control mice.

Table 15. Liver Tumor Incidence in BALB/c Mice Fed Three Dose Levels Technical DDT Over a Lifetime (Terracini et al., 1973).

Dose	Number of Animals[1]			Liver Tumors[2]		
ppm	P/F1 Male	P Female	F1 Female	P/F1 Male	P Female	F1 Female
0	107	62	69	2 (2%)	0	0
2	112	63	72	3 (3%)	0	0
20	106	61	67	1 (1%)	1 (2%)	0
250	106	63	58	15 (14%)	28 (44%)	43 (74%)

[1] Total number of animals necropsied.
[2] Number of animals with liver tumors, percent in parentheses.

The study was fully described including histological methods and findings. Since mortality was higher in treated males and in females of the highest dose group after 80 weeks, the lack of adjustment by the authors for intercurrent mortality makes the interpretation of the incidence data difficult, particularly for male mice. Statistical tests were not described. It was suggested that the greater tumorigenic impact observed in the F1 generation mice may have been caused by the prenatal exposure and DDT intake through nursing.

Tomatis et al. (1972) and Turusov et al. (1973) fed DDT contaminated diets to six generations of CF1 mice. Five dose levels of 0, 2 ppm (0.3 mg/kg/day), 10 ppm (1.5 mg/kg/day), 50 ppm (7.5 mg/kg/day), and 250 ppm (37.5 mg/kg/day) were given to the mice beginning at 6 to 7 weeks of age, and treatment was continued to 140 weeks of age for the parental generation and to 130 weeks for the succeeding generations. The control diet was found to be accidentally contaminated; the parental generation received 0.78 ppm DDT for 6 weeks. Otherwise, the control diet contained 0.02 to 0.09 ppm DDT. Gamma BHC was present on two occasions at 0.01 ppm and 0.04 ppm. No other organochlorine pesticides or aflatoxins were detected. Survival was lower in males and females in the 250 ppm dose group. Mortality in males was also higher in the other DDT treated groups after 70 weeks but did not appear to be related to DDT treatment in females. DDT intoxication, manifested as tremors and convulsions followed by death, was observed in the 50 and 250 ppm dose groups. The average age at death in all mice ranged between 91 and 100 weeks in the control and three lower dose groups but was 79 weeks in males and 82 weeks in females in the 250 ppm group. The excess mortality in treated mice was concluded to be caused by the presence of hepatomas since age-specific death rates among hepatoma-free mice were not different between treatment groups and control. Body weight was greater in the treated mice compared to controls in the parental and F1 generations. Data on growth were not reported for succeeding generations.

The percent tumor bearing mice in the treated males (89 - 94%) was higher than in male controls (78%). DDT treated females had an incidence (85 - 90%) similar to that of the female controls (89%). Liver tumor incidence was increased by DDT treatment in a dose-related manner. These data are presented in Table 16. Liver tumor incidence was significantly greater in treated males at all dose levels ($p<0.001$) (except in the F3 and F5 generations) and in treated females at the 50 ($p<0.01$) and 250 ppm ($p<0.001$) dose levels. Hepatomas in DDT treated males occurred at an earlier age than in control males at all dose levels and occurred earlier in treated females in the 250 ppm group. Liver tumors appeared earlier in the F1 through F5 generations compared to the parental generation in the two higher dose groups, but the incidence did not increase progressively with consecutive generations. Hepatoblastomas were also significantly increased in DDT treated male mice at 10, 50, and 250 ppm. Hepatoblastomas were present in 3 (1%) male controls, and in 5 (1.4%), 14 (3.9%), 12 (3.1%), and 25 (7.1%) of the males in the 2 ppm, 10 ppm, 50 ppm and 250 ppm dose groups, respectively. Of the 56 hepatoblastomas found in the DDT treated groups, 10 (18%) metastasized to the lung. Two metastasized to the lung in the 10 ppm dose group, two metastasized in the 50 ppm dose group, and six metastasized in the 250 ppm dose group. The 3 hepatoblastomas in the controls did not metastasize. The incidence of other tumor types was not related to DDT treatment.

Table 16. Hepatoma Tumor Incidence in CF1 Mice Fed Four Dose Levels Technical DDT For a Lifetime Over Six Generations (Tomatis et al., 1972; Turusov et al., 1973).

Dose ppm	Hepatoma Tumor Incidence[1] by Generation						
	P	F1	F2	F3	F4	F5	Total
				Males			
0	13/55	12/58					97/328
	24%	21%	34%	35%	26%	39%	30%
2	25/58	32/66					179/354
	44%	48%	62%	50%	57%	42%	51%
10	28/53	24/51					181/362
	53%	47%	56%	60%	40%	44%	50%
50	24/53	43/74					214/383
	45%	58%	69%	60%	53%	47%	55%
250	38/50	44/53					301/350
	76%	85%	91%	89%	95%	80%	86%
				Females			
0	2/56	2/55					16/340
	5%	4%	2%	3%	7%	8%	5%
2	3/56	1/49					12/339
	5%	2%	9%	9%	0%	0%	4%
10	2/59	9/65					32/355
	3%	14%	14%	5%	8%	10%	9%
50	7/55	6/49					43/328
	13%	12%	11%	15%	16%	11%	13%
250	31/49	29/41					192/293
	61%	71%	51%	67%	80%	64%	66%

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[1] Ratio of the number of animals with liver tumors to the number of animals surviving to the time the first tumor was observed. Percent of surviving animals.

The study design and experimental results were thoroughly reported. A decided tumorigenic effect by DDT in CF1 mice was indicated at a dose as low as 2 ppm in males. The study is of good quality because sufficiently large samples were used in all dose groups, and animals were followed for their lifespan. Tumor incidence was adjusted for differential survival in treatment groups, and results were tested for statistical significance. The spontaneous incidence of liver tumors was relatively high in male mice of all generations (24 - 39%), but was quite low in female mice (2 - 8%). Although the incidence in parental controls may have been lower if the diet had not been contaminated, tumor incidence in control mice in the other generations fed an uncontaminated diet was higher.

Tomatis et al. (1974) also conducted an experiment to evaluate the effect of the length of exposure on liver tumor incidence in CF1 mice. Nine to ten week old mice, 60 per sex per dose group, were fed 250 ppm DDT for 15 weeks or 30 weeks and autopsies were performed at the cessation of exposure, and at 65, 95, and 120 weeks after the beginning of treatment. A group of control mice were killed at the same time periods.

The incidence of hepatomas is presented in Table 17. The limited DDT exposure still resulted in an increased and early appearance of hepatomas in treated mice. Incidence was lower in mice exposed for the shorter time period however. The size and multiplicity of the hepatomas were correlated with the duration of exposure and time of autopsy. The percent tumor incidence did not decrease over time which indicates that the tumors did not regress but continued to grow after treatment was discontinued.

Table 17. Incidence of Hepatomas in Mice Fed DDT at 250 ppm for 15 or 30 Weeks and Sacrificed at Various Times (Tomatis et al., 1974).

	Percent of Male (M) and Female (F) Mice With Hepatomas Week Animal Was Killed									
	15		30		65		95		120	
Dose Group	M	F	M	F	M	F	M	F	M	F
Control	0	NA	NA	0	11%	0	27%	0	26%	1%
15 Weeks	0	0	NA	NA	21%	5%	43%	18%	41%	10%
30 Weeks	NA	NA	0	0	63%	8%	71%	18%	66%	23%

NA = Not Applicable

A later experiment treated Swiss mice (30 mice/sex/group) beginning at 6 weeks of age for 80 weeks with 100 ppm technical DDT orally, by intubation (10 mg/kg), dermally (0.25 mg), and subcutaneously (0.25 mg) (Kashyap et al., 1977). No significant difference was reported between the survival rates of the control and the experimental groups, and nearly 50% of all animals were alive at 80 weeks. Body weight and growth rate were similar between groups, but signs of DDT intoxication were frequently noted in the groups dosed by oral intubation and subcutaneous injection after 40 weeks of exposure.

Slight increases in liver tumor incidence were reported in the exposed groups but were not significantly different from the control mice except in mice dosed subcutaneously. The liver tumors appeared only after 40 weeks. Lung adenomas and lymphomas were significantly increased compared to controls in the mice dosed in the diet and via oral intubation. Males and females were equally susceptible in this study. The shorter length of this study and smaller number of mice per dose group did not allow the complete ascertainment of the carcinogenic potential of the DDT tested.

A National Cancer Institute study (1978) found no treatment-related effect on tumor development in B6C3F1 mice fed technical DDT for 78 weeks. The mice (50/sex/group treated, 20/sex/group nonexposed) were fed doses of DDT which were changed over the 78 weeks and were then switched to the control diet and observed for an additional 15 weeks. Time weighted average doses of 148 ppm (3.3 mg/kg/day males, 13.05 mg/kg/day females) and 261 ppm (6.6 mg/kg/day males, 26.25 mg/kg/day females) were reported. DDT treatment was significantly associated with increased mortality in females. Survival was also poor in control and treated male mice.

The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens cautioned that the results of the study should be viewed in light of the study's shortcomings. These included the small matched control groups, the fact that the study was conducted in a room where other chemicals were being tested, the numerous dosage changes during the course of the chronic study, and the variations in the pathology protocol.

The NCI study also evaluated the carcinogenic potential of DDE and DDD in mice. Average dose rates of DDE in males and females were 22.2 mg/kg/day and 39.15 mg/kg/day. Treated females experienced a significantly greater mortality rate compared to controls, and both control and DDE dosed male mice had poor survival. Hepatocellular carcinomas were significantly increased in the treated males and females (see Table 18.). DDD was not associated with the development of tumors in either sex.

Table 18. Incidence, after 93 Weeks of Observation, of Hepatocellular Carcinomas in B6C3F1 Mice Fed Two Dietary Dose Levels of DDE for 78 Weeks (NCI, 1978).

Dose Mg/Kg/Day	Number of Animals Male[1]	Number of Animals Female[2]	Hepatocellular Carcinoma[3] Male	Hepatocellular Carcinoma[3] Female
0	8	19	0	0
22.2	38	47	7 (18%)	19 (40%)**
39.15	36	48	17 (47%)*	34 (71%)**

[1] Number surviving to 52 weeks.
[2] Number necropsied.
[3] Number and percent of total.
* $p = 0.013$
** $P < 0.001$

DDE and DDD were evaluated in CF1 mice by Tomatis et al. (1974). A dietary concentration of 250 ppm (37.5 mg/kg/day) p,p'-DDE and/or p,p'-DDD was fed to 6 to 7 week old mice (60 animals/sex/group), and treatment was continued to 130 weeks of age. A total of 90 females and 100 males were fed a control diet that was found to contain DDE and DDD concentrations ranging between 0.01 and 0.06 ppm. The survival rates for the DDE treated mice were similar to controls until week 60, however at week 90, only 10% of the DDE treated mice had survived. The survival rates for DDD treated mice were similar to the controls. Body weight gain was similar for all groups. Histopathological methods and results were reported, but no statistical analysis was performed.

DDE exposure resulted in a higher incidence and earlier appearance of liver tumors (see Table 19). Liver tumors increased from 1% in controls to 98% in treated females. Incidence in males was 74% in dosed animals compared to 34% in controls. Animals treated with DDD showed slight increases in liver tumor incidence in males, and lung adenomas were greatly increased in both sexes. A high percent of spontaneous lung tumors were experienced in male (54%) and female (41%) controls.

Table 19. Incidence of Liver and Lung Tumors in CF1 Mice Fed 250 ppm DDE and/or DDD to 130 Weeks of Age (Tomatis et al., 1974).

Dose Group	Number of Animals[1]		Lung Tumors Number/Percent		Liver Tumors Number/Percent	
	Male	Female	Male	Female	Male	Female
Control	98	90	53 (54%)	37 (41%)	33 (34%)	1 (1%)
DDE	53	55	19 (36%)	9 (16%)	39 (74%)	54 (98%)
DDD	59	59	51 (86%)	43 (73%)	31 (52%)	1 (1%)

[1] Survivors at the first tumor observed.

An early experiment using Osborne-Mendel rats started weanling rats on treated diets of 0, 200, 400, 600, and 800 ppm technical DDT and continued treatment for two years (Fitzhugh and Nelson, 1947). These concentrations corresponded to a dose rate of 0, 10, 20, 40, and 80 mg/kg/day. Tremors were observed particularly in mice in the 600 and 800 ppm dose groups. Tremors developed earlier in females. Treatment had no effect on food intake, however growth reduction (statistically significant) was reported at 12 weeks in females dosed at 400 ppm and in males dosed at 800 ppm. At 12 months growth was retarded at all dose levels in females, and the decrease was significant between 200 and 400 ppm. Only slight retardation in growth was reported for males in the 600 and 800 ppm dose groups. A dose-related increase in mortality was observed in females at all concentrations. The mortality rate was not dose-related in males.

Although the sex and dose group of tumor bearing animals was not reported, the authors concluded that DDT showed a minimal tendency to cause the formation of hepatic tumors. Of the 75 rats surviving to 18 months, 15 had large adenomas (4) or ademonatous hyperplasia (11), with a difference only of size between the two tumor types. The spontaneous hepatic tumor formation rate in similarly aged historical controls was reported to be 1%. This study was not well reported and is not suitable for use in a quantitative risk assessment. However, the results do suggest a tumorigenic effect in rats by DDT.

Liver tumors were induced in Wistar rats through the administration of dietary concentrations of 500 ppm technical DDT (35 mg/kg/day) from 7 weeks of age to 152 weeks of age (Rossi et al., 1977). The animals, 34 - 36 rats/sex/dose group, experienced a slight retardation of growth that was related to treatment. In addition, more than 80% of females had tremors from the age of 16 weeks and had to be fed a standard diet for 7 to 14 days to "normalize their behavior." A few of these rats died. Survival rates in treated and control groups were similar.

The number of tumor bearing rats as a percent of the number of survivors at the time the first tumor was observed was increased in treated rats compared to controls. The incidence in treated and control males was 70.4% and 54.3%, respectively. Incidence in treated and control females was 82.1% and 59.4%, respectively. A greater incidence of nodular liver tumors (neoplastic nodules) was observed in the treated males and females (see Table 20). The total incidence of other neoplasms was higher in controls than in treated animals, but the difference was not statistically significant and was not consistently higher for specific types. The data were not evaluated statistically. Liver tumors in males occurred between 100 to 130 weeks of age. In females, four liver tumors developed before 99 weeks, while the remainder occurred between 100 and 130 weeks of age. The number and size of the neoplastic nodules increased with age. The study design was adequate to detect a treatment-related increase in tumor incidence and histopathologic methods and observations were well reported.

Table 20. Neoplastic Nodules in Wistar Rats Fed 35 Mg/Kg/Day Technical DDT for 145 Weeks (Rossi et al., 1977).

Dose Group	Number Animals[1]		Liver Tumors (# and %)	
	Male	Female	Male	Female
Control	35	32	0	0
Treated	27	28	9 (33%)	15 (54%)

[1] Survivors at time of first tumor observed in each group.

Cabral et al. (1982) also reported a small but statistically increased incidence of liver tumors in Wistar rats (30 - 38 rats/sex/dose) fed 0, 125, 250, and 500 ppm DDT for 144 weeks. These concentrations corresponded to a dose rate of 0, 6, 12, and 24 mg/kg/day. Survival rates of the treated groups were similar to the controls. A slight depression, not significant, in body weight was noted in both treated females and males. Liver tumor incidence is presented in Table 21. The tumors were classified as incidental or fatal and were stated to be analyzed statistically according to the methods proposed by Peto et al. Liver tumors appeared late in life and in greater incidence in females. A significant positive trend for liver tumor incidence was noted in females ($p < 0.001$, Cochran-Armitage test for trend). The incidence of tumors in other organs were comparable in treated and control animals.

Table 21. Liver Tumor Incidence in Wistar Rats Fed Three Dose Levels Technical DDT for 144 Weeks (Cabral et al., 1982).

Dose Group ppm	Number Animals		Liver Tumors (#/%)	
	Male	Female	Male	Female
0	38	38	1 (2.6%)	0
125	30	30	0	2 (6.6%)
250	30	30	1 (3.3%)	4 (13.3%)
500	38	38	2 (5.2%)	7 (18.4%)

This study was well conducted and well reported. Although a relatively small sample size was used, liver tumors were produced in a dose-related manner in female Wistar rats treated over a lifetime.

Six other studies of carcinogenicity in rats reported no treatment-related effects. Concentrations of 0.36, 3.6, and 36 mg/kg DDT dissolved in arachis oil were given to albino rats six days per week, for 60 weeks (Cameron and Cheng, 1951). This dose rate was stated to be equivalent to a dietary concentration of 3.5, 35 and 350 ppm. The number of rats of each sex dosed per group was not stated. Histological evaluation revealed that two female rats had developed centrolobular necrosis, but no significant differences were noted between treated rats and controls. Only six rats were killed at the end of the experiment. The authors stated that a number of animals died at one stage in the study because of an epidemic of pasteurellosis, and 12 of the rats died from lipoid pneumonia and pulmonary edema attributed to the method of dosing. The study design, as well as the disease events which occurred during the experiment, caused this study to be insensitive to the detection of tumors in the rats.

Carworth rats, 40 animals/sex/dose group, were fed 2.5, 12.5, and 25 ppm DDT for two years (Treon and Cleveland, 1955). Data were presented indicating that treatment did not increase mortality, shorten the lives of the animals, or affect the rates of growth. Males in the highest dose group and females in the lowest dose group were reported to have significantly increased liver weight to body weight ratios compared to controls after 18 months or two years, but the data were not presented. Although pathologic methods were not described, characteristic degenerative changes in hepatic cells were reported in treated rats. Tumor incidence was not discussed. The study design and methods used in the experiment were not described thoroughly, and since tumorigenicity was not discussed, it is difficult to draw conclusions regarding the study's results in this area. The maximum tolerated dose (MTD) was not used in this study causing its sensitivity for the detection of tumors to be low.

Ramdoski et al. (1965) exposed 30 Osborne-Mendel rats of each sex to 80 ppm DDT in their diet for two years. Growth, survival, and mortality were reported to be unaffected by treatment. Although the data were not presented, cumulative mortality at the conclusion of 24 months of feeding was stated to be 65% for the males and 60% for the females. The incidence of malignant and benign tumors was not different in treated rats from controls. Undifferentiated bronchogenis carcinoma was noted in five out of six treated females with malignant tumors compared to one out of six control females. Three out of four treated males with malignant tumors also had this cancer compared to one out of three males. The dose level administered to the rats may have been too low to allow the detection of tumors with the sample size used.

Deichmann et al. (1967) also reported no treatment-related increase in tumor incidence in Osborne-Mendel rats fed DDT for 116 weeks. A concentration of 200 ppm DDT (10 mg/kg/day), given to 30 males and 30 females, did not affect survival or growth of the treated rats compared to controls. Percent survival at 24 months was 50% and 83% in treated males and females, respectively, and 50% and 60% in control males and females, respectively. Experimental methods were described and pathological descriptions were thorough. One treated male and 9 treated females were found to have tumors, whereas 1 control male and 13 control females had tumors. Only one liver tumor was observed in a treated female. The majority of the tumors in control and treated females were benign mammary tumors. Since the MTD was not used in this study and the sample size was small, the ability to detect a significantly increased tumor incidence in treated rats may have been low.

Fisher rats (15 males and 15 females) were dosed 15 mg DDT by gavage, five times per week for one year (Weisburger and Weisburger, 1968). The animals were observed for an additional 6 months. Average length of survival was 14 months. No liver tumors were produced, however the sample size and length of exposure and observation causes this study to be insensitive to the tumorigenic properties of DDT.

The National Cancer Institute (1978) evaluated the effect of the long-term feeding of DDT, DDE, and DDD to Osborne-Mendel rats. A total of 50 rats per sex per dose group were used, except that only 20 rats per sex were used in the control group. The animals were dosed for 78 weeks during which time the dietary concentration was changed. The low and high time weighted average doses of DDT reported were 16.05 mg/kg/day (male) and 10.5 mg/kg/day (female) and 32.1 mg/kg/day (male) and 21 mg/kg/day (female), respectively. Low and high dose levels of DDD were 82.4 mg/kg/day (males) and 42.5 mg/kg/day (females) and 164.7 mg/kg/day (males) and 85 mg/kg/day (females), respectively. Low and high dose levels of DDE were 21.85 mg/kg/day (males) and 12.1 mg/kg/day (females) and 41.95 mg/kg/day (males) and 23.1 mg/kg/day (females) respectively.

No evidence of carcinogenicity was found for DDT or DDE in either sex at the given doses, although increased mortality was seen in both sexes of rats dosed with DDE. Although a significant increase in tumors was not observed in rats fed DDE, the substance did induce centrolobular necrosis and fatty metamorphosis. Males in the low dose group for DDD developed a significantly increased incidence of follicular cell adenomas and carcinomas of the thyroid (16 out of 49 treated (33%) vs 1 out of 19 controls (1%); $p = 0.016$). Thyroid tumors were not significantly increased in the higher dose group. Therefore the results were determined to be only suggestive of a DDT related effect. A dose-related mean body weight depression was noted in males and females fed DDD, but treatment was not related to increased mortality. The limitations of the study were discussed previously, and in combination with the less than lifetime duration of observation, allow only the determination that the results were suggestive of a tumorigenic effect.

Long-term bioassays in Golden Syrian hamsters have consistently resulted in negative results for carcinogenicity for DDT. However, one study which tested the effects of DDE reported and increase in tumor incidence among treated hamsters.

Syrian golden hamsters, 30 per sex per dose, were fed 500 and 1000 ppm DDT in their diets for 48 weeks (Agathe et al., 1970). Treated hamsters showed a slight decrease in survival and growth rates. No significant treatment-related increase in tumor incidence was reported, although the observation period was less than one year. Incidence in control animals was 14% (5 out of 36 necropsied) compared to 12% (7 out of 58) in the 500 ppm dose group, and 8% (4 out of 52) in the 1000 ppm dose group.

Graillot et al. (1975) administered a dietary concentration of 250, 500, and 1000 ppm DDT to groups of 30 male and 30 female hamsters for 18 months. Growth rates in the exposed group were similar to those in the controls, the length of survival in the treated hamsters was longer. Lymphosarcomas were the only tumors reported, and the incidence of these tumors was reduced in the treated animals compared to controls. The length of observation in the study was relatively short.

The negative response of hamsters to the administration of DDT in the diet was confirmed by two later studies. Cabral et al. (1982) fed groups of hamsters diets containing 0, 125, 250, and 500 ppm DDT over their lifetimes. Survival and growth were not significantly different between dose groups. Tumor incidence, summarized in Table 22, was evaluated in relation to the number of survivors at the time of appearance of the first tumor.

Table 22. Tumor Incidence in Syrian Golden Hamsters Fed Three Dose Levels of DDT For Their Lifetimes (Cabral et al., 1982).

Dose ppm	Number of Animals[1] M	Number of Animals[1] F	Number of Tumors Pituitary M	Pituitary F	Adrenal M	Adrenal F	Liver M	Liver F	Other M	Other F
0	40	39	0	2	3	0	0	0	0	3
125	30	28	0	0	4	0	0	0	3	3
250	31	28	0	0	6	1	3	0	2	1
500	39	40	0	2	8	3	0	0	3	8

[1] Number of survivors at the time of appearance of the first tumor.

Although a dose-related increase in adrenal tumors was observed in males and females, incidence between treated groups and controls was not significantly different. This study was well conducted and thoroughly reported.

A higher dose level of DDT fed to Syrian Golden hamsters over the lifetime of the animals also did not produce tumors (Rossi et al., 1983). Dietary levels of 1000 ppm DDT produced no significant differences in survival between treated hamsters and controls, however a body weight reduction was noted (not significant). DDE fed to groups of hamsters at concentrations of 500 and 1000 ppm in the diet, also caused differences in body weight gain that were not significant. Hamsters treated with DDE had a significantly higher incidence of liver tumors compared to controls (see Table 23.). These tumors appeared late in life and were classified as neoplastic nodules. The first liver tumor appeared in females exposed to 1000 ppm DDE at 76 weeks and in males in the highest dose group at 105 weeks. The first liver tumors were observed in females fed 500 ppm at 94 weeks and in males at 105 weeks.

Table 23. Tumor Incidence in Syrian Golden Hamsters Fed Two Dose Levels of DDE For a Lifetime (Rossi et al., (1983).

Dose ppm	Number Animals Male	Number Animals Female	Liver Tumors (# and %) Male	Liver Tumors (# and %) Female
0	10	31	0	0
500	15	26	7 (47%)[a]	4 (15%)[c]
1000	24	24	8 (33%)[b]	5 (21%)[d]

[1] Number of survivors at time of the observation of the first liver tumor.

[a] $p = 0.017$
[b] $p = 0.013$
[c] $p = 0.048$
[d] $p = 0.01$

The finding that DDE, but not DDT, produces tumors in hamsters suggests that the DDT metabolite is the active agent for tumorigenicity in hamsters. This is consistent with the limited ability of this species to metabolize DDT to DDE (Gingell and Wallcave, 1974).

Two studies evaluated the effects of DDT exposure in rhesus monkeys (Durham et al., 1963). A total of 24 adult rhesus monkeys of both sexes were studied, 22 of which were fed DDT and 2 were fed DDE. Nine monkeys served as controls. Concentrations of 5 ppm (5 males), 50 ppm (4 males, 1 female), 200 ppm (5 males, 5 females), and 5000 ppm (1 male, 1 female) DDT and 200 ppm DDE were fed to the animals for a period of up to 7.5 years. Four of the animals on the 50 ppm DDT diet were later placed on a diet containing 5000 ppm DDT.

No signs of illness consistent with DDT poisoning were reported for monkeys exposed to 200 ppm DDT or less, and no effect on food consumption or body weight was observed. Animals fed 5000 ppm DDT experienced decreased food consumption and rapid weight loss, and all monkeys developed tremors, convulsions, and other signs of DDT poisoning. No changes in liver histopathology were reported for monkeys dosed with 200 ppm and less based on periodic biopsies or autopsies. Six animals which had survived for 7.5 years were continued on treatment. Therefore, a complete ascertainment of their status regarding liver tumors was not possible.

Adamson and Sieber (1983) administered 20 ppm DDT to rhesus monkeys by gavage five days per week for 11 years. The five monkeys that died were not found to have developed tumors. The apparent cause of death in these animals was concluded to be DDT-induced central nervous system toxicity. The 19 surviving monkeys appeared to be normal with no evidence of tumor formation based upon biopsies and no changes in biochemical parameters. A complete ascertainment of tumors could not be obtained without autopsies however. Autopsies were not conducted on all exposed and control animals, and the length of observation may not have been long enough for tumors to develop in the rhesus monkeys. Therefore the lack of tumor development in these studies cannot be viewed as conclusive.

Conclusion

The epidemiologic data are inadequate to draw conclusions concerning the carcinogenicity of DDT in humans. One case-control study (Flodin et al., 1988) indicated a possible association of DDT with leukemic lymphoma, however more retrospective research will be required to confirm the relationship. Other studies of DDT manufacturing workers and pesticide applicators suggest elevated lung cancer mortality. All individuals in the study populations were exposed to several pesticides however, and a longer period of follow-up is required to establish any mortality trends (Ditraglia et al., 1981; Wong et al., 1984; Barthel, 1981; Wang and MacMahon, 1979; Blair et al., 1983). There have been some negative epidemiologic findings, but these studies were not designed to detect cancer in the populations studied.

A careful evaluation of the long-term feeding studies in exposed mice, rats, hamsters and monkeys results in the conclusion that DDT is carcinogenic in mice and rats. DDE is carcinogenic in mice (Tomatis et al., 1974) and hamsters (Rossi et al., 1983), while DDD exposure resulted in a possible increase in thyroid tumors in rats (NCI, 1978). DDT treatment resulted in a consistent negative response in four studies of hamsters, however DDE produced liver tumors in this species (Rossi et al., 1983). Studies of monkeys exposed to DDT have not indicated a tumorigenic effect, however these results were not conclusive. The organ affected most often is the liver. Significantly increased lung tumors and lymphomas have also been reported in DDT treated rodents.

Of those well-conducted studies which evaluated survival and were able to present tumor incidence in relation to the number of animals alive at the time the first tumor was identified in each group, liver tumors were significantly increased in both males and females (Thorpe and Walker, 1973; Turusov et al., 1973). Female mice were affected to a greater degree than males in one study (Thorpe and Walker, 1973), In another study the increase in incidence was comparable. Turusov et al. (1973) reported a 56% increase in liver tumor incidence in males fed 250 ppm technical DDT for a lifetime compared to the incidence in controls, while the proportion of females with liver tumors treated with the same concentration was 61% higher than the proportion in controls. The increase in males was statistically significant at a lower dose level in males, however, and males also had a significantly increased incidence of malignant hepatoblastomas beginning at 10 ppm DDT. Both studies used CF1 strain mice.

Females may be more sensitive to the tumorigenic effect of DDE. Tomatis et al. (1974) reported a 98% incidence of liver tumors in female CF1 mice fed 250 ppm DDE at 130 weeks of age compared to 1% in control females. Incidence in treated males was 74% compared to 34% in male controls. Liver tumor incidence in Wistar strain rats was higher in DDE treated females than treated males (Rossi et al., 1977; Cabral et al., 1982).

DDT and DDE treatment caused liver tumors to appear at an earlier age in mice (Thorpe and Walker, 1973; Terracini et al., 1973; Turusov et al., 1973; Tomatis et al., 1974) and in rats (Cabral et al., 1982). Liver tumors were also identified earlier in DDE exposed mice (Tomatis et al., 1974).

Other nonhepatic tumors have been associated with exposure to DDT in laboratory animals. Innes et al. (1969) reported a significantly increased incidence of malignant lymphoma in females of one of the two strains of mice studied (C57Bl/6 x AKR cross). An increase in lung tumors was also noted but the incidence was not statistically significant. An increased incidence of leukemias and pulmonary carcinoma was significant in BALB/c mice fed 2.8 to 3.0 ppm p,p'-DDT over six generations (Tarjan and Kemeny, 1969). In contrast, the incidence of lymphomas in another group of BALB/c mice was lower in the highest dose group (250 ppm) compared to the control and two lower dose groups (Terracini et al., 1973). This result may have been due to intercurrent mortality in the high dose group, however. A lower tumor incidence (not significant) was reported in the lung, lymph tissue, and kidney in treated mice in the Walker and Thorpe study. Possible explanations for the lower tumor incidence have not been identified, although intercurrent mortality may have been responsible in part.

An increased incidence of liver tumors was induced in male mice by a concentration of 2 ppm in the diet over a lifetime (Tomatis et al., 1972; Turusov et al., 1973). A dose-response relationship was observed in the studies using three or more dose groups above 50 ppm in mice (Walker et al., 1972). Liver tumor incidence did not appear to be different between 2 ppm and 20 ppm (Terracini et al., 1973; Turusov et a., 1973). Cabral et al. (1982) demonstrated an increasing liver tumor incidence in rats with increasing dose in rats between 125 and 500 ppm.

Mice exposed to DDT for less than a lifetime developed a higher incidence of liver tumors than controls after a maximum of 120 weeks of observation (Tomatis et al., 1974). Incidence was lower in mice exposed for 15 weeks than in mice exposed for 30 weeks. The percent tumor incidence reached a maximum at 95 weeks and remained essentially the same at 120 weeks in males. In females, incidence continued to increase slightly in mice treated for the longer period, while the percent of animals with tumors dosed for 15 weeks had decreased from 18% to 10% by 120 weeks. The size and multiplicity of the hepatomas were correlated with the duration of exposure and time of autopsy. In summary, even when exposure to DDT was curtailed, liver tumor incidence was greater in exposed animals compared to controls at the end of their lives.

Although several studies reported no increase in tumor incidence in mice, rats, or monkeys exposed to DDT or DDE, they all suffered from problems in study design or the occurrence of unintended events which rendered the experimental results inconclusive. These limitations included small sample size, imprecise dose estimates, incomplete follow-up of survivors, inadequate length of follow-up, and the use of a dose level much lower than the maximum tolerated dose for that species.

Five of the eight positive studies in mice are considered to be of sufficient quality to provide evidence that DDT causes liver tumors in male and female mice (Innes et al., 1969; Thorpe and Walker, 1973; Terracini et al., 1973; Turusov et al., 1973), lung adenoma and lymphoma in both sexes of mice (Kashyap et al., 1978), and malignant lymphoma in female mice (Innes et al, 1969). Although some of these experiments used only one dose level, less than 50 animals/sex/dose, or involved less than lifetime exposure or followup, they all showed a statistically increased incidence of tumors at one or more sites in males and females. In addition, methods were fully described, survival was evaluated, and the tumor incidence data were presented by site and by sex. Two studies are judged to be suggestive of tumorigenicity for leukemia and pulmonary carcinoma (Tarjan and Kemeny, 1969; Shabad et al., 1973), and one study is suggestive for liver tumors (Walker et al., 1972). These studies were limited because survival was not evaluated or mortality was very high, tumor incidence was presented for all animals only, not for each sex, and statistical methods were not used.

An adequately conducted bioassay in Wistar rats fed technical DDT observed a statistically significant dose-related trend in liver tumor incidence in females (Cabral et al., 1982). Rossi et al. (1977) produced an increased incidence of neoplastic nodules in the livers of male and female Wistar rats but did not evaluate the data statistically. The results of a study by Fitzhugh and Nelson (1947) are suggestive of the tumorigenic capability of

DDT. The tumors induced in rats were benign. In addition, rats appeared to be less sensitive to the carcinogenic properties of DDT because tumors were observed at relatively high dose levels.

Four studies of DDT exposure in hamsters resulted in a negative finding (Agathe et al., 1970; Graillot et al., 1975; Cabral et al., 1982; Rossi et al., 1983). Two of the studies were well conducted and well reported. This negative evidence is consistent with the inability of this species to metabolize DDT to DDE.

Administration of DDE to mice caused an enhanced development of liver tumors in two studies (NCI, 1978; Tomatis et al., 1974). The NCI study suffered from various limitations including small matched control groups, potential exposure to other chemicals, numerous dose changes during the course of the study, and variations in pathology protocol. Therefore the evidence of carcinogenicity observed in DDE treated mice is viewed as suggestive. The Tomatis et al. study was thoroughly reported and the study design was adequate. Although tumor incidence was not evaluated statistically, the increase in liver tumor incidence in DDE treated mice compared to controls was quite large.

DDE has also been determined to be tumorigenic in male and female Golden Syrian hamsters (Cabral et al., 1983). Although tumor incidence was elevated in males, an increase with dose level was not observed. However, the increase was statistically significant at both dose levels used, and the discrepancy in males may have been due to the small number of animals alive at the time of the first observation of the first liver tumor in the dose groups. A dose-related increase in incidence was observed in females.

MECHANISMS OF ACTION FOR CANCER

MUTAGENICITY

The mutagenic capability of DDT and its metabolites has been studied extensively in microorganisms and mammalian cell lines. Generally the studies of point mutations and unscheduled DNA synthesis have not indicated an ability to cause mutations in genetic material. On the other hand, DDT was positive in a test of dominant lethality in *D. melanogaster*. An association with mammalian chromosome damage has also been determined in several species.

Studies of mutagenic activity in bacterial test systems and eukaryotic yeast cells were reviewed in the U.S. EPA Ambient Water Quality Criteria Document for DDT (1980). DDT and DDE did not increase the frequency of reversions in *Salmonella typhimurium* strains TA-1535, 1537, 98, or 100 using DDT concentrations of 4 ug/plate to 2500 ug/plate. The frequency of reversions was also not increased by DDE at a concentration of 1000 ug/plate. Activation with S-9 microsomes did not alter the results. The growth of *E. coli* Pol-A strains was not inhibited at 500 ug DDT, DDE, or DDD. DDT and metabolites, tested in several other bacterial systems, have not caused evidence of point mutations.

DDD produced mutations in a host mediated assay. Buselmaier et al. (1972) administered DDD to mice and assayed for back mutation of *Salmonella typhimurium* and *E. marcescens* following incubation in the peritoneum. Although administration of DDD resulted in back mutations, the administration of DDT, DDE, and DDA did not have this effect.

Tests of DDT and metabolites have not produced genotoxic results in eukaryotic cells. Single strand breaks in DNA were not produced in a study of mitotic gene conversion in *Saccharomyces cerevisiae*. Genotoxic results were not produced in host mediated studies using cells from rat testis, liver, and lung. No significant increases in mutations in the conidia of *Neurospora crassa* incubated *in vitro*, and in the host mediated assay were observed.

DDT and DDA were found to have mutagenic activity in a study of X-linked recessive lethal mutations in *Drosophila melanogaster*, but DDE, DDD, and DDOH did not. A dominant lethal test of DDT in *D. melanogaster* found that sequential breeding of the treated males with virgin females at three day intervals resulted in an increase in dominant lethality in early spermatid and spermatocyte stages. The lethal effect was correlated with an increase in nondisjunction.

Changes in chromosome structure, chromosome aberrations, and chromatid aberrations have been caused in *in vitro* assays using mammalian cell lines. Palmer et al. (1972) reported that p,p'-DDT, o,p'-DDT, p,p'-DDE, p,p'-DDD and o,p'-DDD at concentrations of 10 - 50 ug/ml caused chromosome abnormalities in an established kangaroo rat cell line. The p,p'- isomers caused a two-fold greater rate of chromosomal aberrations than the o,p'- isomers. Treatment of p,p'-DDA was cytogenic only at 200 ug/ml. Kelly-Garvert and Legator (1973), Bradley et al. (1981), and Ray-Chaudhuri et al. (1982) found that DDE caused an increase in chromosome aberrations in a Chinese hamster V79 cell line at concentrations of 30 and 35 ug/ml. The majority of the chromosome aberrations were composed of exchange figures and chromatid breaks. DDT was not clastogenic in this cell line at the concentrations studied. DDT, DDD, and DDE, but not DDA, caused chromosome breakage and gap formation in a B14F28

Chinese hamster cell line (Mahr and Miltenburger, 1976). No chromosomal structural anomalies were observed.

Structural and chromosomal aberrations were reported in exposed human lymphocytes. Lessa et al. (1976) found a significantly greater proportion of cells with structural aberrations in lymphocytes exposed to 0.2, 4.05, and 8.72 ug/ml DDT compared to controls, however concentrations of 12 and 15 ppm produced no effect. The lowest doses administered, 0.06 to 0.2 ug/ml (ppm) were stated to be similar to those found in the plasma of individuals of the general population in Brazil.

Rabello et al. (1975) compared the frequency of leucocytes with chromosomal aberrations in 50 workers from three insecticide plants' (A, B, and C) in Sao Paulo, Brazil. Twenty-five workers had direct contact with DDT. The mean age of these workers was 25, and their mean exposure time was 2 years, 4 months (range 2 months to 10 years). Total DDT and DDE levels in the plasma of the workers was 0.16 to 3.25 ug/ml (ppm) total DDT (mean 1.03 ug/ml ± 0.79) and 0.03 to 1.77 ug/ml (ppm) p,p'-DDE (mean 0.48 ± 0.52). The other twenty-five workers were not exposed directly to DDT. Their mean age was 31 and mean exposure time was 2 years, 15 days (range 1 month to 19 years). Plasma levels in this group ranged from 0.03 to 1.46 ug/ml (ppm) total DDT (mean 0.38 ug/ml ± 0.15) and 0.01 to 0.41 ug/ml (ppm) p,p'-DDE (mean 0.15 ± 0.02).

Upon analysis, these two groups were not found to differ in the frequencies of cells with chromosomal or chromatid aberrations. In one plant however, five individuals in the indirectly exposed group were found to have high plasma DDT levels and were included with the directly exposed workers. This newly defined group of exposed workers had a significantly higher frequency of leukocytes with chromatid gaps and breaks when compared to the remaining controls. A second sample of 8 workers with a mean DDT plasma level of 0.24 ug/ml did not have significantly increased chromatid aberrations when compared to control subjects from the general population (mean DDT plasma level 0.03 ug/ml). This was a very small sample size however. The authors concluded tentatively that exposure to DDT causes chromatid aberrations.

Preston et al. (1981) also reported an increase in chromosomal aberrations in human lymphocyte cultures exposed to DDT. DDT or its metabolites produced no effect on unscheduled DNA synthesis in exposed human fibroblasts, or rats, mouse, and hamster hepatocytes.

In vivo studies of mutagenesis in mammals have produced positive and negative results. Two oral doses of 150 mg/kg technical DDT administered to male Swiss mice caused dominant lethal mutations in the early spermatid and spermatocyte stages (Clark, 1974). A reduction in the number of live implants per female, and an increase in the number of dead implants was observed. Chronic oral doses of DDT (100 mg/kg, twice a week, for 10 weeks) caused a persistent increase in the number of dominant lethal mutations. Chronic dosing caused significant reductions in sperm viability and a reduction of cell numbers in all stages of spermatogenesis. Three other studies of dominant lethality in mice were negative (Epstein and Shafner, 1968; Buselmaier et al., 1972; Palmer et al., 1973).

Two studies reported chromosomal damage in DDT treated mice, however a similar study in rats was negative. Johnson and Jalal (1973) gave single i.p. injections to groups of BALB/c mice at a DDT concentration of 100, 150, 200, 300, or 400 mg/kg body weight. A significant increase in the number of chromosome fragments in bone marrow cells was noted in mice dosed 150 mg/kg and higher. A significantly increased number of sticky cells was noted at all concentrations. Larsen and Jalal (1974) administered i.p. doses to BALB/c mice ranging from 25 to 250 mg/kg. Chromosomal deletions, and gaps plus deletions were significantly higher in the bone marrow of mice dosed with 50 mg/kg or higher compared to the controls. In another study, doses ranging from 20 to 100 mg/kg/body weight administered by i.p or by gavage to rats did not show a dose-response relationship or an increase in the percent of chromosomal aberrations over the controls (Legator et al., 1973).

STUDIES OF PROMOTING ACTIVITY

Several studies of the promoting activity of DDT have been conducted using various initiation-promotion systems in rats, mice, and hamsters. DDT has been found to enhance the production of neoplasms in all systems investigated except in the hamster.

The promoting activity of DDT was first described by Peraino et al. (1975) using 2-acetylaminofluorene (AAF)-induced hepatic tumorigenesis in the Sprague-Dawley rat. Male Sprague-Dawley rats, 22 days of age, were fed either control diets or diets containing 0.02% AAF for 18 days. All rats then received the control diet for 7 days. Rats originally fed the control diet were continued on the untreated feed (48 rats/group). The remainder were then divided into five groups and fed either the control diet (120 rats/group) or a diet containing 0.05% of one of four agents for the duration of the experiment. The test agents were phenobarbital, amobarbital, diphenylhydantoin, or DDT. The authors calculated the average daily intake of the test chemicals to be 50 mg/kg body weight for the first four to six months and then approximately 20 mg/kg body weight by month 15. No symptoms of toxicity or sedation were reported, however body weight gain was not discussed.

Rats fed DDT without prior exposure to AAF had significantly increased liver weight per gram body weight at 270 days of treatment (3.89, S.E=0.16 vs 3.16, S.E.=0.08, $p < 0.001$). Phenobarbital had a similar effect. Centrolobular hypertrophy was also observed at all sacrifice intervals in the rats exposed to these two test agents. Tumors were not observed in these rats after 389 days. However, the number of rats per group were stated not to be sufficient to adequately test carcinogenic potential.

The administration of AAF plus DDT or phenobarbital caused a higher percentage of rats bearing tumors and a larger average number of tumors per liver compared to AAF controls at each sacrifice interval. By 390 days, it was reported that greater than 90% of the rats bore tumors, and had 2 to 2.5 tumors per liver. The incidence in the AAF only controls was 50% with 0.5 to 1.0 tumors per liver. The incidence increased in the AAF plus DDT or phenobarbital exposed groups for the first 250 days after the last AAF treatment. After 250 days a plateau was observed. The incidence in AAF controls did not plateau. Therefore the authors concluded that a longer observation period would be needed to determine if DDT or phenobarbital treatment increased the incidence of total tumors over AAF alone rather than causing an earlier appearance of tumors. No consistent relationship was reported in the AAF control group for the relative incidence of adenomas to carcinomas. An increased proportion of rats in the AAF plus DDT or phenobarbital groups were observed to have adenomas at all time intervals. The overall incidence of carcinomas in AAF treated rats was not enhanced by chemical treatment except at 378 or 442 days in animals fed DDT. The authors concluded that the classification of DDT as a tumor enhancer may be appropriate because of its correspondence with the action of phenobarbital.

Scribner and Mottet (1981) using a similar protocol, tested the promotion of tumors induced by 2-acetamidophenanthrene (AAP) in male Sprague-Dawley rats (20 rats per dose group). Dose levels of 0.02% AAP in the diet and 0.05% DDT were used. DDT feeding was discontinued after 45 days, and the animals were observed a total of 60 - 65 weeks.

The authors were particularly interested in evaluating the promotion of hepatic tumors induced by AAP. AAP is not a hepatocarcinogen but is mutagenic in *Salmonella typhimurium* and forms nucleic acid adducts in an amount similar to that obtained from AAF, a potent hepatocarcinogen. Liver tumors were not observed in the rats exposed to AAP plus DDT or in those exposed to the two agents alone.

Rats fed AAP (with and without DDT) developed invasive ear-related tumors and mammary gland tumors (21/40). Exposure to DDT enhanced the incidence of these tumors in the rats. By 38 weeks, cumulative tumor incidence was 68% in rats fed AAP plus DDT and was 21% in the rats exposed to initiating treatment of AAP ($p < .001$). The number of AAP plus DDT treated rats with tumors was greater than the number of rats fed AAP only with tumors at each sacrifice interval. A log-rank statistical evaluation of the difference between the two tumor induction curves was statistically significant ($p=.008$). Both tumor types appeared earlier in DDT treated rats induced by AAP. A greater number of DDT treated rats had one or the other tumor types than AAP induced (without

DDT) rats at every time interval up to the end of the 65 week study period. One tumor, a benign subcutaneous fibroma, was detected in the rats exposed to DDT alone at 68 weeks. The role of DDT as a potential promoter of human female breast cancer was suggested.

The promoting ability of DDT has also been investigated in diethylnitrosamine (DEN) treated rats. Nishizumi (1979) administered 50 ppm DEN in drinking water to 200 male, 28 day old, Wistar rats for two weeks. After one week on the control diet, groups of rats were given one or a combination of three test agents. These were 500 ppm phenobarbital sodium in drinking water, 0.1 ml 1.25% DDT administered by gastric intubation, 0.1 ml 10% Kanechlor 500 administered by gastric intubation, or a combination of two or three of the compounds. In each group, one-half (6 - 10) of the rats were killed 40 weeks and one-half (7 - 9) were killed 52 weeks after the beginning of the experiment.

DDT did not promote DEN initiated liver tumors. The administration of DEN plus DDT produced 0.2 ± 0.2 and 0.4 ± 0.2 tumors larger than 5 mm at 40 weeks and 52 weeks, respectively. DEN alone resulted in zero tumors at these time periods. The combination of DEN, DDT and PCB resulted in a significant reduction in the number of tumors compared to treatment with DEN plus PCB at 40 weeks (1.0 ± 0.5 vs 3.3 ± 0.7, $p < 0.05$). The number of tumors was also reduced at 52 weeks, but not significantly (4.4 ± 1.6 vs 6.9 ± 2.1).

Body weight and liver weight were not affected by DDT in DEN-induced rats. In addition, the average number of liver tumors, 5 mm or larger, per rat were not increased in this group over the rats treated only with DEN at 40 weeks (0.2, S.E.=0.2 compared to 0) or at 52 weeks (0.4, S.E.=0.2 compared to 0).

DDT promoted tumors induced by DEN in male B6C3F1 mice (Williams and Numoto, 1984). Exposures were begun at eight weeks of age whereupon DEN was provided to groups of mice (approximately 40 mice per group) at 20 ppm in the drinking water for 14 weeks. Other groups were left untreated to be used as a control, or to evaluate the effect of chemical treatment alone. After a four week interval, 200 ppm N-2-fluorenylacetamide (FAA), 25 or 50 ppm technical chlordane, 5 or 10 ppm technical heptachlor, or 50 ppm DDT was administered via the diet for 25 weeks. The total study duration was 43 weeks. Randomly selected mice were killed at four time intervals and the remainder at the end of the study. The effects of the chemicals on liver foci and the development of neoplasms were determined in comparison to the groups receiving only DEN or chemicals without DEN.

After 25 weeks of exposure to the test chemicals, survival was decreased in mice exposed to DEN, but the reduction was not statistically significant. Body weight was reduced in all the treated mice and was greater than 10% less than that of the untreated control. Liver weight as a percent of body weight was not affected in DDT treated mice compared to the appropriate control group.

The characteristic lesion in DEN induced foci at 25 weeks after cessation of DEN exposure was a decrease in G-6-Pase activity. The number of DEN-altered foci in DEN plus DDT treated mice was 3.08 profiles/cm^2 (S.E.=2.82) compared to 1.27 profiles/cm^2 (S.E.=1.07) in the DEN only group although the enhancement was not statistically significant. The percent area covered by the altered foci was also larger than the DEN control, but the increase in area was not significant (0.78, S.D.=1.2 vs 0.13, S.D.=0.12). The incidence and areas of altered foci in mice treated only with DDT was similar to that in the untreated controls.

The incidence of DEN-induced neoplasms was enhanced by DDT treatment, but the increase was not significant (66.7% compared to 40.0% in DEN controls). Multiplicity of neoplasms per liver were also increased over controls (1.14, S.D.=1.17 vs 0.65, S.D.=1.01). Mice treated only with DDT had no neoplasms, while untreated controls had an incidence of 10.7%. A greater number of adenomas were produced than carcinomas at each time interval, but DDT treatment appears to have increased the proportion of carcinomas induced by DEN. DEN treatment alone resulted in 2 carcinomas out of 13 neoplasms while DEN plus DDT resulted in 10 carcinomas out of 24 neoplasms. Other neoplasms caused by DEN (squamous cell papillomas and carcinomas in the forestomach and pulmonary adenomas) were not enhanced by the chemicals indicating an organ specific promoting action.

The promotion and inhibition of enzyme-altered islands initiated by 3′-methyl 4-(dimethylamino)-azobenzene (3′-Me-DAB) was studied by Kitagawa et al. (1984). The study was designed to determine a dose of DDT which did not promote tumors but did exert anticarcinogenic action. The study protocol involved feeding male Donyru rats, 21 days old, a diet containing 600 ppm 3′-Me-DAB for three weeks and then a diet containing phenobarbitol (PB) or DDT at various doses. At 12 weeks of age, five rats/group were killed and at 24 weeks of age, 6 to 10 rats/group were killed. The study of simultaneous exposure involved feeding male Donyru rats and Fisher rats a diet containing 100 ppm 3′-Me-DAB and various doses of PB or DDT, respectively. Animals were killed at 12 weeks (5 rats/group) and at 24 weeks of age (4 - 5 rats/group). The size and number of ATP-ase deficient islands were measured.

DDT exerted a dose-related effect on the number and size of enzyme altered islands (EAI). The number of EAI was significantly increased in the 5, 20, 50, 100, and 500 ppm dose groups ($p<0.01$). The increase was not significant in the 10 ppm group. The number of large EAI were significantly increased in the higher dose groups. DDT administered simultaneously with 3'-Me-DAB at concentrations of 50 and 100 ppm also enhanced the number of EAI produced at 12 (0.08 ± 0.05 and 0.12 ± 0.15 EAI/cm^2) and 24 weeks (2.2 ± 0.76 and 2.1 ± 0.54 EAI/cm^2). DDT was therefore both a promoter at relatively low doses, and a co-carcinogen when the initiating dose of 3'-Me-DAB was low. DDT administered alone at a dose of 500 ppm resulted in the production of 0.21 ± 0.25 EAI/cm^2 at 24 weeks of age. This number is comparable to that caused by 3'-Me-DAB alone (0.17 ± 0.04 EAI/cm^2). The authors concluded that their original goal of finding a practical threshold level for DDT was not feasible.

Neither DDT or phenobarbital promoted dimethylnitrosamine (DMN)-initiated hepatocarcinogenesis in hamsters (Tanaka et al., 1987). Groups of 11 to 15 animals were administered 6 mg/kg body weight DMN by intraperitoneal injection and then fed the control diet for one week. One group of 15 hamsters were continued on the basal diet for 30 weeks, while three others were fed 500 ppm phenobarbital in the diet for 30 weeks, 500 ppm DDT in the diet for 30 weeks, or carbon tetrachloride by gavage every two weeks for 30 weeks. Another three groups were treated with the three test chemicals using the same protocols without prior DMN administration, and one group was maintained on the basal diet only for 31 weeks.

Body weights and liver weight (per 100 g body weight) were not affected by chemical treatment except by CCl_4 after DMN administration. DMN treatment caused the appearance of a high number of altered foci compared to the untreated controls, but only CCl_4 treatment enhanced the number and area of DMN induced foci. All three chemicals administered without DMN caused a significantly larger number of altered foci compared to the untreated control. Only CCl_4 enhanced the number of hamsters with DMN-initiated liver neoplasms and the number of neoplasms per animal. DDT administered alone caused enlarged hepatocytes with fatty metamorphosis in the central or midzonal regions and periportal fibrosis. Although DDT and phenobarbital alone both caused the appearance of altered foci, the authors found it difficult to explain why these agents did not enhance the DMN-induced foci. It was concluded that a species difference in effect was indicated.

DDT has been tested in assays which evaluate the inhibition of intercellular communication and metabolic cooperation, believed to be common attributes of promoters (Malcolm and Mills, 1985; Trosko et al., 1981). DDT has responded consistently in these assays.

Klaunig and Ruch (1987) used primary cultured hepatocytes from four strains of male mice and one strain of male rats to evaluate the effect of DDT on intercellular communication. Hepatocytes were isolated from B6C3F1, C3H/He, C57Bl, and BALB/c mice, and F344 rats. "Donor" hepatocytes were labelled with [5-^{3}H] uridine. Recipient hepatocytes obtained from a second animal were plated on the donor cells, and phenobarbital, DDT or dieldrin (dissolved in 0.01% DMSO) were added to the cultures. Autoradiography was used to detect recipient cells in direct contact with donor cells. Cells in direct contact had an increase in grain distribution compared with isolated cells. Intercellular communication was defined as cells having twice the number of grains compared with each of three isolated recipient cells. Triplicate cultures were examined for each treatment and strain. The use of primary hepatocyte cultures has advantages for the study of xenobiotics that may exert their effects through activated metabolites.

DDT inhibited intercellular communication between B6C3F1 mouse hepatocytes at 1, 5, and 10 ug/ml and between C3H mouse, C57BL mouse, BALB/c mouse and F344 rat hepatocytes at 5 and 10 ug/ml (Table 24). The data indicate that the inhibition is dose-related. The authors stated that these concentrations were not cytotoxic or inhibitory of hepatocyte [^{3}H] RNA synthesis.

Table 24. Effect of DDT on Intercellular Communication in Cultured Hepatocytes from Mice and Rats After 8 Hour Treatment.[a]

	Percent Communicating Recipients[b]				
Treatment	B6C3F1	C3H	C57BL	BALB/c	F344
No Treatment	83.5 (5.1)	84.6 (3.5)	89.6 (1.2)	93.2 (1.3)	83.0 (2.4)
DMSO 0.1%	85.8 (3.4)	84.6 (1.0)	88.3 (1.2)	90.3 (3.6)	82.1 (3.4)
DDT 1 ug/ml	72.2 (5.4)*	79.1 (3.1)*	89.1 (4.0)	84.6 (1.0)	79.7 (3.1)
DDT 5	66.6 (3.2)*	69.7 (2.1)*	78.2 (2.2)*	77.6 (1.5)*	72.6 (1.1)*
DDT 10	64.3 (2.1)*	63.9 (2.0)*	77.9 (0.9)*	77.4 (3.6)*	68.5 (6.5)*

[a] Adapted from Klaunig and Ruch, 1987.

[b] Values represent the mean and standard deviation (in parentheses) of the percent of communicating primary recipients in triplicate cultures per treatment group in which at least 100 primary recipient hepatocytes were evaluated per culture.

* Values were significantly decreased ($p < 0.05$) vs DMSO solvent control group (Student's t-test).

In another study, DDT decreased the frequency of communicating recipients in a dose-related manner using the same protocol and six month old B6C3F1 mice hepatocytes (Ruch et al., 1987). Eight hours after the addition of DDT to the medium, the percent of communicating recipients was significantly less than the DMSO control at each of the four nontoxic concentrations used (0.1, 0.5, 1.0, and 5.0 ug/ml). The authors suggested that a lipophilic compound such as

DDT may partition into the plasma membrane lipid and affect intercellular communication by altering gap junction alignment.

DDT was also shown to inhibit metabolic cooperation in V79 Chinese hamster lung fibroblast cell lines (Warngard et al., 1987). The V79 metabolic cooperation assay measures the survival of mutant V79 6-thioguanine resistant (HG-PRT$^-$) cells plated with wildtype V79 6-thioguanine sensitive (HG-PRT$^+$) cells in the presence of the test chemical and selecting agent, 6-thioguanine (Malcolm and Mills, 1985). HG-PRT$^+$ cells have the enzyme, hypoxanthine-guanine phosphoribosyl transferase (HGPRTase), which catalyses the conversion of preformed purine bases (hypoxanthine and guanine) into their respective nucleotides. HGPRTase also catalyses the conversion of certain abnormal bases, such a 8-azaguanine and 6-thioguanine. When these abnormal bases are incorporated into nucleic acid in these cells, they exert a cytotoxic effect on the cells. Mutant cells are deficient for the enzyme and therefore will survive in the presence of 6-TG unless they are in direct contact with HGPRT$^+$ cells, and gap junctions have formed. A chemical is classified as an inhibitor of metabolic cooperation if the recovery of mutants is significantly increased compared to the solvent controls at noncytotoxic concentrations. Because V79 cells have a limited capacity to metabolize chemicals, observed responses are assumed to result from the effects of unmetabolized test substances.

DDT caused a dose-dependent increase in the percent recovery of mutant colonies beginning at a concentration of 5 uM. The increase was statistically significant beginning at 10 uM DDT. A study of the interactive effect of the addition of 0.1 nM of the tumor promoter TPA to increasing concentrations of DDT resulted in an increasing dose-dependent percent recovery of mutants that was significantly higher than the calculated additive response. When DDT (10 uM) was added to increasing concentrations of TPA, a similar synergistic response was observed except at the highest concentration tested. At this concentration, the recovery of mutants was not significantly higher than the calculated additive response. The authors concluded that the synergism of TPA and DDT support an assumption that tumor promoters exert their action through different pathways.

Metabolic cooperation is implicated in developmental signalling, embryonic induction, growth control, cell nutrition, the control of hormonal responses, and general tissue homeostasis (Malcolm and Mills, 1985). This connection, and the finding that many tumor promoters as well as several complete carcinogens inhibit metabolic cooperation *in vitro*, has led researchers to hypothesize that inhibition of metabolic cooperation may be a mechanism for tumor promotion in adults and for teratogenesis in developing embryos. Others have pointed out however that tumor promoters *in vivo* exhibit organ specific activity whereas *in vitro* assays demonstrate that tumor promoters of diverse chemical nature inhibit intercellular communication in a variety of cell types (Ruch et al., 1987). DDT demonstrates this capacity. Therefore other factors *in vivo* such as pharmacokinetics and metabolism may be important mediators of tumor promotion.

WEIGHT OF EVIDENCE CLASSIFICATION

U.S.EPA's cancer guidelines state that an increased incidence of only mouse liver tumors will be regarded as sufficient evidence of carcinogenicity if all other conditions for a classification of "sufficient" evidence in animal studies are met. Factors that would lower the evidence classification to "limited" include lack of replication, increased incidence at high dose only or at the end of an experiment, no dose-related increase in malignancies, predominantly benign responses, no dose-related shortening of the time to the appearance of tumors, negative or inconclusive results from a spectrum of short term tests for mutagenic activity, the occurrence of excess tumors only in a single sex.

The U.S. EPA's Science Advisory Board held a workshop on the relevance of mouse liver tumors and rat kidney tumors for predicting carcinogenicity in humans on August 12, 1987. The SAB concluded that, "for the most part, mouse liver tumors are indicative of human carcinogenicity" (Cothern, 1987). The supporting evidence, discussed at the workshop, is summarized below.

1. Mouse liver is the most common target site in the National Toxicology Program bioassays for a variety of chemicals. Although male mice have a high spontaneous rate of liver tumors, liver tumors in female mice is a more common target site. Female mice have a very low spontaneous incidence of liver tumors.
2. Mouse liver is one of the most common target sites in animals for compounds on the IARC list of potential human carcinogens, classified on the basis of epidemiologic studies and animal studies (not necessarily liver cancer in humans).
3. The pathology of mouse liver tumor resembles those in other species, including humans; and it goes through a sequence of histogenesis which is predictable, at least with spontaneous tumors, and those types of tumors such as induced by DEN. A typical liver carcinoma in the mouse will metastasize to the lungs similarly to liver tumors in all other species including humans.
4. Mouse liver responds to well recognized carcinogens in a dose-related fashion similarly to the response of other organs to carcinogens.

5. Multiplicity can be very important especially with increased incidence in one sex, in one strain of one species.
6. About 70% of chemicals that cause liver tumors in mice cause other kinds of tumors in mice or rats; and up to 70% of those mouse liver carcinogens are also mutagens.
7. The more organs that are involved and, the more species that are involved, the more convincing is the evidence a chemical has carcinogenic potential in humans.

The epidemiologic data are inadequate to draw conclusions concerning the carcinogenicity of DDT in humans. One case-control study (Flodin et al., 1988) indicated a possible association of DDT with leukemic lymphoma, however more retrospective research will be required to confirm the relationship. Other studies of DDT manufacturing workers and pesticide applicators suggest elevated lung cancer mortality. However, all individuals in the study populations were exposed to several pesticides, and a longer period of follow-up is required to establish any mortality trends (Ditraglia et al., 1981; Wong et al., 1984; Barthel, 1981; Wang and MacMahon, 1979; Blair et al., 1983). There have been some negative epidemiologic findings, but these studies were not designed to detect cancer in the populations studied.

The evidence of carcinogenicity in mice and rats exposed to DDT are sufficient to place this persistent compound into the U.S. EPA weight of evidence classification of B2, a probable human carcinogen. Tumor incidence was significantly increased in five different strains of mice. Tumors were produced in replicate studies in two mouse strains, although the target organ in BALB/c mice was not always the liver. All but two of the studies in mice reported both benign and malignant liver tumors. Turusov et al. (1973) and

Tomatis et al. (1972) reported that the "hepatomas" occasionally metastasized to the lungs. These authors also reported that DDT treatment also increased the multiplicity of tumors per tumor-bearing animal.

Other nonhepatic tumors have been associated with exposure to DDT in laboratory animals including malignant lymphoma in female mice, lung adenoma and lymphoma in male and female mice, and leukemia and pulmonary carcinoma in male and female mice.

The liver was the only target organ for carcinogenicity in rats. The three studies that reported liver tumors used three different rat strains. The liver tumors were benign and appeared late in life, suggesting that DDT is a less potent carcinogen in rats compared to mice. A dose-response relationship was observed by Cabral et al. (1982). Many studies have demonstrated the ability of DDT to promote cancer in several experimental systems and DDT has been shown to have co-carcinogenic properties as well.

The studies of DDE exposure in mice and hamsters are less extensive. A significant increase in hepatocellular carcinoma was induced in male and female mice in a dose-related manner (NCI, 1978). Tomatis et al. (1974) reported a significant increase in both benign and malignant liver tumors with metastases to the lungs in four treated mice and two control mice. A larger percentage of treated mice had tumors greater than 10 mm in diameter, and liver tumors appeared at an earlier age. In hamsters, the liver tumors were classified as neoplastic nodules and occurred late in life. Neoplastic nodules were absent in the control hamsters. Although significantly increased in both low and high treatment groups, incidence was dose-related in females, but not in males. The tumors occurred at an earlier age in the high dose group in females, but no difference between dose groups was observed in males. DDE should be placed in the same weight of evidence classification as DDT because it is a metabolite of DDT and evidence of its carcinogenic potential exists in mice and hamsters.

The U.S. EPA (1986) concluded that the evidence for the carcinogenicity of DDT in mice was adequate. The agency determined that the evidence in rats was limited because of the higher dose levels required, the benign nature of the tumors, the lack of an increase in the total number of tumor bearing animals, and the lack of DDT-induced mortality. DDT was still categorized as a B2 carcinogen because of limited evidence of its ability to cause cancer in trout, the existence of evidence for positive mutagenicity, and the ability of DDT to promote cancer.

DOSE RESPONSE ASSESSMENT

SYSTEMIC TOXICITY

The evidence available to date identifies the liver as the most sensitive target for chronic systemic toxicity. Several studies observed microscopic alterations in hepatic cells and increased activity of hepatic microsomal enzymes in rats fed 0.25 mg/kg/day for several months (Nelson et al., 1949; Laug et al., 1950; Ortega et al., 1956; Kinoshita et al., 1966; Gillett et al., 1966). This dose level has been determined to be the lowest adverse effect level (LOAEL) for liver toxicity in rats.

No histopathologic changes were found in rats fed 0.05 mg/kg/day by Laug et al. (1950). This is determined to be the no adverse effect level (NOAEL) for liver toxicity in rats. Hepatic microsomal enzyme induction has been reported in rats exposed to this dose level (Kinoshita et al., 1966). Moreover, elevated enzyme activity was indicated in a few DDT manufacturing workers studied by Laws et al. (1973). The men were exposed to an estimated 0.05 mg/kg/day to 0.26 mg/kg/day. This dose rate may be in the range of the threshold for enzyme induction in humans. Enzyme induction is an indication of exposure to chlorinated hydrocarbons, but it is not clear that this is an adverse effect.

The data on reproductive toxicity and developmental toxicity in the literature are not sufficient to determine a NOAEL or LOAEL for use in developing a reference dose for human exposure to DDT. More research is needed, particularly studies of behavioral effects. Scudder and Richardson (1970) demonstrated a decrease in aggression in the male offspring of mice exposed to 0.0002 mg/kg/day technical DDT in drinking water. Behavioral effects should be carefully studied in additional laboratory species and in highly exposed human populations.

Calculation of the Reference Dose (RfD)

Table 25 presents the Reference Dose (RfD) for noncarcinogenic toxicity, and the NOAEL, uncertainty factors (UF), and modifying factor (MF) upon which it is based. The RfD applies to DDT, DDD, and DDE because the exact form of the chemical responsible for toxicity has not been determined. Two reference doses were calculated for each study. The RfD(total) represents the "allowable" dose from all exposure sources. The RfD(fish) represents the "allowable" dose from fish obtained by sport fishing. This was determined by subtracting the dose of DDT/DDE from all other environmental sources from the RfD(total). The dose from all other sources for DDT/DDE is 0.00004 mg/kg/day. This estimate is derived from the U.S. FDA market basket survey for 1981-82

(Gartrell et al., 1986) which measured residue levels in a typical adolescent male diet and the assumption that approximately 90% of the total DDT/DDE dose is derived from food (see section on human exposure).

Table 25. Parameters Used to Calculate the Reference Dose (RfD) for Systemic Effects in Humans Exposed to DDT, DDD, and DDE.

Reference	Target Organ	Dose mg/kg/day	End-point	Study Length months	UF[2]	MF[3]	Reference Dose mg/kg/day Total	Reference Dose mg/kg/day Fish
Laug et al., 1950	Liver	0.05	NOAEL[1]	6	10H 10A	1	0.0005	0.00046

[1] NOAEL = No Adverse Effect Level

[2] UF = Uncertainty factor. Uncertainty factors are used to calculate the reference dose. The no-adverse-effect-level (NOAEL) is divided by factors of 10 to account for variation in sensitivity among the members of the human population, 10H; and uncertainty in extrapolating from animal data to humans, 10A. (See Table 5, Methods and Scientific Rationale for Hazard Assessment, Dose Response Assessment, and Risk Characterization for details.

[3] MF = Modifying factor. An additional uncertainty factor, greater than zero and less than or equal to 10, to further adjust for scientific uncertainties of the study.

Calculation of the Potency Factor (q*) for Carcinogenicity

Potency factors (95% upperbound) were calculated using the linearized multistage mathematical model for carcinogenesis, and the specific parameters and assumptions presented in Table 26.

Mice are the most sensitive species to the carcinogenic effect of DDT and DDE. The chronic study reported by Turusov et al. (1973) contained the data on cancer incidence selected for the calculation of the potency factor. Several factors recommend this study for use in a quantitative risk estimation. The study design and experimental results were thoroughly reported. Several doses were tested, sufficiently large samples were used in all dose groups, and animals were followed for their lifespan. Tumor incidence was adjusted for differential survival in treatment groups and results were tested for statistical significance. Tumor incidence increased with increasing dose in both males and females. The data for cancer incidence in male mice did not adequately fit the linearized multistage model as indicated by rejection using the Chi-Square Goodness of Fit test, even when the model was run without including the highest dose group. Therefore the potency factor ($q^* = 0.39$) was calculated from the data in females.

The two studies which evaluated DDE in mice are not satisfactory for use in a quantified risk estimation. The NCI study (1978) did not adjust for survival differences in addition to its other problems which were discussed previously. Tomatis et al. (1974) used only one dose level. This study was adequately reported in other respects, and therefore, the potency factor, $q^* = 2.54$, was calculated as a sort of worst case check for DDE.

The U.S. EPA (1986) calculated a potency factor for DDT/DDD/DDE by taking a geometric mean of potency factors calculated from a variety of studies in mice and rats and using data from both DDT and DDE. This practice, although reasonable in theory, is not appropriate in this case because the studies used were not all of comparable quality. Some aspects of the study designs possibly had a strong effect on the tumor incidence observed. The practice of combining tumor incidence across species is unacceptable. It involves the assumption that human beings fall somewhere between mice and rats in sensitivity to DDT and DDE. Actually, the relative sensitivity of humans is not known.

Table 26. Parameters, assumptions, and resulting potency factors for DDT and DDE.

	Turusov et al., 1973	Tomatis et al., 1974
Species	CF-1 Mice	CF -1 Mice
Sex	Female	Female
Material	Technical DDT	Technical DDT
Dietary Conc. (ppm)	0 2 10 50 250	0 250
% Food Rate	17%	17%
TWA dose rate (mg/kg/day)	0 0.3 1.5 7.5 37.5	0 37.5
Absorption	100%	100%
Length of Exposure	130 weeks	130 weeks
Study Duration	130 weeks	130 weeks
Incidence	P and F1 4/111 Control 4/105 Low 11/124 Med1 13/104 Med2 60/90 High	1/90 Control 54/55 Treated
Potency factor (q^*)	0.39 $(mg/kg/day)^{-1}$	2.54 $(mg/kg/day)^{-1}$

RISK CHARACTERIZATION

Tables in the Appendix to this report present the upper 95% limit on cancer risk and hazard indices for each sport fish species using alternative values for meal size and meal frequency, and the average, minimum, and maximum tissue concentration in fish samples collected in 1985 and 1986. The tissue concentration data for both years were combined when they were available, or data from either year were used when a species was sampled in only one of those years (see exposure assessment section).

The estimates of cancer risk and other health hazards are for a 70 kg adult. It is important to note that children and lighter adults will have a higher DDT/DDE dose rate per meal and are more susceptible to health problems than these estimates indicate. For example, a 13.7 kg (30 lb) toddler eating 12 quarter pound meals of brown trout (all sizes) per year, will have a DDT/DDE intake of 0.00012 mg/kg/day. This dose rate is five times higher than the dose for a 70 kg adult eating the same amount.

Hazard Indices and Contributions to Uncertainty

Tables 27 and 28 show the hazard index for each species for a meal size of 0.228 kg (0.5 lb) and a consumption frequency of one meal per month or two meals per week. At a consumption rate of one meal per month, the hazard indices based on mean tissue concentration are below one for all species and size classes. At a consumption frequency of two meals per week, the hazard indices are less than one for 10 - 20 inch lake trout, 10 - 20 inch brown trout, walleye, chinook less than 30 inches, coho, and perch. Species with hazard indices above one are 20 - 30 inch lake trout, 20 - 30 inch brown trout, and chinook greater than 30 inches. The range in tissue concentration results in a variation in hazard indices of one order of magnitude between the maximum and minimum tissue concentrations for each species. The variability is greater for coho salmon.

Upper 95% Limits on Cancer Risk Estimates and Contributions to Uncertainty

An upper 95% confidence limit for DDT potency of 0.39 $(mg/kg/day)^{-1}$ was estimated from Turusov et al. (1973) for liver tumor incidence in female CF-1 mice. A DDE potency factor (q_1^*) of 2.54 $(mg/kg/day)^{-1}$ was estimated from Tomatis et al. (1974) for liver tumor incidence in female CF-1 mice. Thus the potency factor for DDE is 6.5 times higher than the potency factor for DDT. Tomatis et al. (1974) used only one dose group which may have resulted in a higher q_1^* than one which may have been calculated based on more than one dose group. Therefore it is difficult to compare these two values for relative "potency".

The data on tissue concentration available for evaluation provide figures for total DDT only, representing a mix of DDT, DDE, DDD, and other DDT residues. The cancer risk estimates presented in the appendix are based for practical purposes on the assumption that the q_1^* for DDT and DDE are equivalent. The proportion of total DDT comprised by DDE is an unknown variable. The degree that the potency factor is underestimated, if at all, by using a q_1^* of 0.39$(mg/kg/day)^{-1}$ is also not known.

Turusov et al. (1973) observed both benign and malignant liver tumors, a few of which metastasized to the lungs. The authors did not indicate what proportion of the total incidence was accounted for by malignant tumors. Therefore a comparison of the effect of the proportion of malignant tumors on the value of the potency factor is not possible.

The risk specific dose based on the maximum likelihood estimate (MLE), using the Turusov et al. (1973) data for liver tumor incidence was 1.8 times higher than the risk specific dose based on the upper 95% confidence interval. The MLEs of the risk specific dose (10^{-5}) based on the multistage, weibull, and multi-hit models and the assumption of additive background, were within the same order of magnitude.

The potency estimates were calculated using an assumed interspecies dose equivalency based on surface area. These estimates are about 14 times higher than potency factors calculated based on an assumed interspecies dose equivalency based on body weight. The use of surface area is supported by the evidence suggesting that DDE may be an active metabolite.

The upper 95% limit on cancer risk resulting from the consumption of a 0.114 kg (0.25 lb) meal once a month or once a year are summarized for all sport fish species in Tables 29 and 30. The upper limit for cancer using average tissue concentration for a species (all size classes) ranges from 2.5×10^{-6} in perch to 3.8×10^{-5} in lake trout when eaten once a month for a lifetime. The upper limit for cancer ranges from 2.1×10^{-7} in perch to 3×10^{-6} in lake trout when eaten once a year for a lifetime. Fish species fall into the following rank order from lowest to highest risk: perch, walleye, coho salmon, brown trout, chinook salmon, and lake trout.

Tissue concentration data for certain species showed a rising trend in tissue concentration with increasing length. Therefore it was possible, for those species, to evaluate the change in risk for different size classes. The cancer risk from lake trout consumption increases by a factor of 5 between 10 - 20 inch fish and 20 - 30 inch fish. The upper 95% bound on cancer risk from the consumption of 20 - 30 inch brown trout is greater by a factor of 1.6 compared to 10 - 20 inch brown trout. Consumption of chinook salmon longer than 30 inches corresponds to an upper 95% cancer risk four times higher than the upper bound risk associated with the consumption of 20 - 30 inch chinook.

The range in tissue concentration within a species caused the risk estimates to vary by approximately one order of magnitude for lake trout, brown trout, walleye, and coho salmon. Variability in upperbound risk estimates was highest between the minimum and maximum tissue concentrations for chinook salmon and perch.

Tables 31, 32, and 33 present the number of meals per year that would result in an upper 95% cancer risk of 1×10^{-6}, 1×10^{-5}, and 1×10^{-4}. An upper limit of 1×10^{-6} would allow the consumption of one 0.5 pound meal per year of lake trout less than 20 inches or chinook salmon less than 30 inches. One to two meals of walleye or coho salmon, or four to five meals of perch might be eaten. A smaller meal size would allow at least one meal per year of all species evaluated except lake trout greater than 20 inches or chinook salmon greater than 30 inches at an upperbound risk of one per million exposed individuals. An upper limit risk of 1×10^{-5} or 1×10^{-4} would allow the consumption of one to several meals of all species depending on the size of the meal eaten and the species selected.

Table 27
Comparison of Hazard Indices (HI) for Various Lake Michigan Sport Fish Species Consumed Once a Month With Meal Size 0.228 Kg (0.5 lb). HI for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Hazard.

		Hazard Index	
	RfD =	0.00046 Liver	
Species (Size Class)	Mean Conc.	Max. Conc.	Min. Conc.
Lake Trout (All Sizes)	0.21	0.72	0.03
Lake Trout (10 - 20 inches)	0.05	0.72	0.16
Lake Trout (20 - 30 inches)	0.28	0.72	0.16
Chinook (All Sizes)	0.17	1.09	0.00
Chinook (20 - 30 inches)	0.10	0.12	0.09
Chinook (> 30 inches)	0.41	1.09	0.15
Brown Trout (All Sizes)	0.10	0.30	0.01
Brown Trout (10 - 20 inches)	0.08	0.14	0.04
Brown Trout (20 - 30 inches)	0.12	0.30	0.01
Coho (All Sizes)	0.04	0.16	0.00
Walleye (All Sizes)	0.03	0.11	0.01
Perch (All Sizes)	0.01	0.03	0.00

Table 28
Comparison of Hazard Indices (HI) for Various Lake Michigan Sport Fish Species Consumed Twice A Week With Meal Size 0.228 Kg (0.5 lb). HI for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Hazard.

	HI Tissue Concentration		
Species (Size Class)	Mean	Max.	Min.
Lake Trout (All Sizes)	1.82	6.25	0.24
Lake Trout (10 - 20 inches)	0.46		
Lake Trout (20 - 30 inches)	2.42	6.25	1.41
Chinook (All Sizes)	1.49	9.48	0.00
Chinook (20 - 30 inches)	0.89	1.01	0.77
Chinook (> 30 inches)	3.59	9.48	1.33
Brown Trout (All Sizes)	0.87	2.62	0.12
Brown Trout (10 - 20 inches)	0.67	1.25	0.32
Brown Trout (20 - 30 inches)	1.07	2.62	0.12
Coho (All Sizes)	0.38	1.39	0.04
Walleye (All Sizes)	0.22	0.95	0.06
Perch (All Sizes)	0.12	0.26	0.00

Table 29
Comparison of the Upper 95% Limit on Cancer Risk for Various Lake Michigan Sport Fish Species Consumed Once a Month With Meal Size 0.228 Kg (0.5 lb). Risk for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Risk.

	Upper Limit (95%) on Risk q* =.39 Mg/Kg/Day-1		
Species (Size Class)	Mean	Max.	Min.
Lake Trout (All Sizes)	3.76E-05	1.29E-04	5.01E-06
Lake Trout (10 - 20 inches)	9.61E-06		
Lake Trout (20 - 30 inches)	5.01E-05	1.29E-04	2.92E-05
Chinook (All Sizes)	3.09E-05	1.96E-04	0.00E+00
Chinook (20 - 30 inches)	1.84E-05	2.09E-05	1.59E-05
Chinook (> 30 inches)	7.43E-05	1.96E-04	2.76E-05
Brown Trout (All Sizes)	1.80E-05	5.43E-05	2.51E-06
Brown Trout (10 - 20 inches)	1.38E-05	2.59E-05	6.68E-06
Brown Trout (20 - 30 inches)	2.21E-05	5.43E-05	2.51E-06
Coho (All Sizes)	7.93E-06	2.88E-05	8.35E-07
Walleye (All Sizes)	4.59E-06	1.96E-05	1.25E-06
Perch (All Sizes)	2.51E-06	5.43E-06	0.00E+00

Table 30
Comparison of the Upper 95% Limit on Cancer Risk for Various Lake Michigan Sport Fish Species Consumed Once a Year With Meal Size of 0.228 Kg (0.5 lb). Risk for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Risk.

	Upper Limit (95%) on Risk q* =.39 (Mg/Kg/Day)-1		
Species (Size Class)	Mean	Max.	Min.
Lake Trout (All Sizes)	3.13E-06	1.08E-05	4.18E-07
Lake Trout (10 - 20 inches)	8.00E-07		
Lake Trout (20 - 30 inches)	4.18E-06	1.08E-05	2.44E-06
Chinook (All Sizes)	2.58E-06	1.64E-05	0.00E+00
Chinook (20 - 30 inches)	1.53E-06	1.74E-06	1.32E-06
Chinook (> 30 inches)	6.19E-06	1.64E-05	2.30E-06
Brown Trout (All Sizes)	1.50E-06	4.52E-06	2.09E-07
Brown Trout (10 - 20 inches)	1.15E-06	2.16E-06	5.57E-07
Brown Trout (20 - 30 inches)	1.84E-06	4.52E-06	2.09E-07
Coho (All Sizes)	6.61E-07	2.40E-06	6.96E-08
Walleye (All Sizes)	3.83E-07	1.64E-06	1.04E-07
Perch (All Sizes)	2.09E-07	4.52E-07	0.00E+00

Table 31
A Comparison of Meal Frequencies Resulting in an Upper 95% Limit on Cancer Risk of 1E-06, 1E-05, or 1E-04 For a Meal Size of 0.114 Kg of Various Lake Michigan Sport Fish Species Ranked by Decreasing Level of Risk.

Species (Size Class)	Meal Frequency (/Yr)		
	Based on 1.00E-06 Risk	Based on 1.00E-05 Risk	Based on 1.00E-04 Risk
Lake Trout (All Sizes)	0.64	6.39	63.85
Lake Trout (10 - 20 inches)	2.50	24.99	249.86
Lake Trout (20 - 30 inches)	0.48	4.79	47.89
Chinook (All Sizes)	0.78	7.77	77.66
Chinook (20 - 30 inches)	1.98	19.82	198.16
Chinook (> 30 inches)	0.32	3.23	32.29
Brown Trout (All Sizes)	1.34	13.36	133.65
Brown Trout (10 - 20 inches)	1.74	17.41	174.14
Brown Trout (20 - 30 inches)	1.08	10.84	108.43
Coho (All Sizes)	3.02	30.25	302.46
Walleye (All Sizes)	5.22	52.24	522.43
Perch (All Sizes)	9.58	95.78	957.79

Table 32
A Comparison of Meal Frequencies Resulting in an Upper 95% Limit on Cancer Risk of 1E-06, 1E-05, or 1E-04 For a Meal Size of 0.228 Kg of Various Lake Michigan Sport Fish Species Ranked by Decreasing Level of Risk.

Species (Size Class)	Meal Frequency (/Yr)		
	Based on 1.00E-06 Risk	Based on 1.00E-05 Risk	Based on 1.00E-04 Risk
Lake Trout (All Sizes)	0.32	3.19	31.93
Lake Trout (10 - 20 inches)	1.25	12.49	124.93
Lake Trout (20 - 30 inches)	0.24	2.39	23.94
Chinook (All Sizes)	0.39	3.88	38.83
Chinook (20 - 30 inches)	0.99	9.91	99.08
Chinook (>30 inches)	0.16	1.61	16.40
Brown Trout (All Sizes)	0.67	6.68	66.82
Brown Trout (10 - 20 inches)	0.87	8.71	87.07
Brown Trout (20 - 30 inches)	0.54	5.42	54.21
Coho (All Sizes)	1.51	15.12	151.23
Walleye (All Sizes)	2.61	26.12	261.22
Perch (All Sizes)	4.79	47.89	478.90

Table 33
A Comparison of Meal Frequencies Resulting in an Upper 95% Limit on Cancer Risk of 1E-06, 1E-05, or 1E-04 For a Meal Size of 0.34 Kg of Various Lake Michigan Sport Fish Species Ranked by Decreasing Level of Risk.

Species (Size Class)	Meal Frequency (/Yr)		
	Based on 1.00E-06 Risk	Based on 1.00E-05 Risk	Based on 1.00E-04 Risk
Lake Trout (All Sizes)	0.21	2.14	21.41
Lake Trout (10 - 20 inches)	0.84	8.38	83.78
Lake Trout (20 - 30 inches)	0.16	1.61	16.06
Chinook (All Sizes)	0.26	2.60	26.04
Chinook (20 - 30 inches)	0.66	6.64	66.44
Chinook (>30 inches)	0.11	1.08	10.83
Brown Trout (All Sizes)	0.45	4.48	44.81
Brown Trout (10 - 20 inches)	0.58	5.84	58.39
Brown Trout (20 - 30 inches)	0.36	3.64	36.36
Coho (All Sizes)	1.01	10.14	101.41
Walleye (All Sizes)	1.75	17.52	175.17
Perch (All Sizes)	3.21	32.11	321.14

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NOTE: ADDITIONAL EPIDEMIOLOGIC STUDY

Subsequent to the writing of this draft report, an additional study was published describing the results of an epidemiological investigation of the association of DDT exposure to human cancer, especially respiratory cancer (Austin, H., J. E. Keil, and P. Cole. 1989. A Prospective Follow-Up Study of Cancer Mortality in Relation to Serum DDT. *AJPH* **79**:43-46). The study decribed the overall mortality and the cancer mortality experience of a cohort of adult residents of Charleston, South Carolina, whose serum DDT levels were measured in the mid-1970's and who were then followed prospectively for 10 years. The original cohort was comprised of 919 subjects of which 23% (209) had died after 10 years, 700 were still alive, and 10 were lost to follow-up. The cause of death was classified as listed on the death certificate and also by a medical committee blind to exposure status.

The subjects were divided into terciles according to serum DDT (DDT plus DDE) concentration; 0 - 31 ppb (305 subjects), 31 - 52 ppb (308 subjects), and > 52 ppb (306 subjects). A slight increasing trend in overall mortality occurred with increasing serum DDT, but it was not significant. Relative rates, adjusted for age, gender, race, years of schooling, and cigarette habit, were 1.0, 1.2, and 1.2 in the three groups. There was no consistent increasing trend in cancer mortality with increasing serum DDT concentrations. A slight positive trend in respiratory cancer rate, also not statistically significant, was reported in the three exposure groups. The adjusted relative rates were 1.0, 1.5 (95% CI; 0.5, 4.9), and 1.8 (95% CI; 0.5, 6.2). The significance of this finding is diminished by the observation that the mean levels of serum DDT were nearly identical for persons who had and had not died of respiratory cancer.

When the respiratory cancer mortality of the cohort was compared with that of the United States and of South Carolina, the SMR for respiratory cancer mortality rate was also higher, but the elevated rate was not significantly greater. The SMR was 122 (95% CI; 76, 187) using the U.S. population and was 124 (95% CI; 77, 189) using the South Carolina population.

The authors suggested that the subjects probably had been exposed to DDT for at least 25 years by 1975 because of its use in that region for mosquito control, as household pesticides, and in agriculture since the 1940s. Twenty-five years would have been a sufficient induction period for cancer. However, the authors stated that the study was too small to exclude moderate increases in respiratory cancer mortality attributable to DDT. The upper 95% confidence limit for excess respiratory cancer mortality associated with a doubling of serum DDT was 83%. The results of this study continue to indicate that DDT may be a weak human lung carcinogen, but a definitive conclusion awaits further research.

CHAPTER FIVE APPENDIX

Brown Trout (All Sizes)

DDT Tissue Concentration (85 + 86)	ppm
Mean	0.43
Max.	1.3
Min.	0.06
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000002	0.000006	0.000000
0.114	2	0.000625	0.000004	0.000012	0.000001
0.114	4	0.001249	0.000008	0.000023	0.000001
0.114	6	0.001874	0.000012	0.000035	0.000002
0.114	12	0.003748	0.000023	0.000070	0.000003
0.114	24	0.007496	0.000046	0.000139	0.000006
0.114	52	0.016241	0.000100	0.000302	0.000014
0.114	104	0.032482	0.000200	0.000603	0.000028
0.114	156	0.048723	0.000299	0.000905	0.000042
0.228	1	0.000625	0.000004	0.000012	0.000001
0.228	2	0.001249	0.000008	0.000023	0.000001
0.228	4	0.002499	0.000015	0.000046	0.000002
0.228	6	0.003748	0.000023	0.000070	0.000003
0.228	12	0.007496	0.000046	0.000139	0.000006
0.228	24	0.014992	0.000092	0.000278	0.000013
0.228	52	0.032482	0.000200	0.000603	0.000028
0.228	104	0.064964	0.000399	0.001206	0.000056
0.228	156	0.097447	0.000599	0.001810	0.000084
0.34	1	0.000932	0.000006	0.000017	0.000001
0.34	2	0.001863	0.000011	0.000035	0.000002
0.34	4	0.003726	0.000023	0.000069	0.000003
0.34	6	0.005589	0.000034	0.000104	0.000005
0.34	12	0.011178	0.000069	0.000208	0.000010
0.34	24	0.022356	0.000137	0.000415	0.000019
0.34	52	0.048438	0.000298	0.000900	0.000042
0.34	104	0.096877	0.000595	0.001799	0.000083
0.34	156	0.145315	0.000893	0.002699	0.000125

Brown Trout (All Sizes) continued.

Upper Limit (95%) on Risk q* =.39 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =2.54 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
7.48E-07	2.26E-06	1.04E-07	4.87E-06	1.47E-05	6.80E-07
1.50E-06	4.52E-06	2.09E-07	9.75E-06	2.95E-05	1.36E-06
2.99E-06	9.05E-06	4.18E-07	1.95E-05	5.89E-05	2.72E-06
4.49E-06	1.36E-05	6.26E-07	2.92E-05	8.84E-05	4.08E-06
8.98E-06	2.71E-05	1.25E-06	5.85E-05	1.77E-04	8.16E-06
1.80E-05	5.43E-05	2.51E-06	1.17E-04	3.54E-04	1.63E-05
3.89E-05	1.18E-04	5.43E-06	2.53E-04	7.66E-04	3.54E-05
7.78E-05	2.35E-04	1.09E-05	5.07E-04	1.53E-03	7.07E-05
1.17E-04	3.53E-04	1.63E-05	7.60E-04	2.30E-03	1.06E-04
1.50E-06	4.52E-06	2.09E-07	9.75E-06	2.95E-05	1.36E-06
2.99E-06	9.05E-06	4.18E-07	1.95E-05	5.89E-05	2.72E-06
5.99E-06	1.81E-05	8.35E-07	3.90E-05	1.18E-04	5.44E-06
8.98E-06	2.71E-05	1.25E-06	5.85E-05	1.77E-04	8.16E-06
1.80E-05	5.43E-05	2.51E-06	1.17E-04	3.54E-04	1.63E-05
3.59E-05	1.09E-04	5.01E-06	2.34E-04	7.07E-04	3.26E-05
7.78E-05	2.35E-04	1.09E-05	5.07E-04	1.53E-03	7.07E-05
1.56E-04	4.71E-04	2.17E-05	1.01E-03	3.06E-03	1.41E-04
2.33E-04	7.06E-04	3.26E-05	1.52E-03	4.60E-03	2.12E-04
2.23E-06	6.75E-06	3.11E-07	1.45E-05	4.39E-05	2.03E-06
4.46E-06	1.35E-05	6.23E-07	2.91E-05	8.79E-05	4.06E-06
8.93E-06	2.70E-05	1.25E-06	5.81E-05	1.76E-04	8.11E-06
1.34E-05	4.05E-05	1.87E-06	8.72E-05	2.64E-04	1.22E-05
2.68E-05	8.10E-05	3.74E-06	1.74E-04	5.27E-04	2.43E-05
5.36E-05	1.62E-04	7.47E-06	3.49E-04	1.05E-03	4.87E-05
1.16E-04	3.51E-04	1.62E-05	7.56E-04	2.28E-03	1.05E-04
2.32E-04	7.02E-04	3.24E-05	1.51E-03	4.57E-03	2.11E-04
3.48E-04	1.05E-03	4.86E-05	2.27E-03	6.85E-03	3.16E-04

Brown Trout (All Sizes) continued.

Hazard Index RfD = 0.00046 Liver Mean Conc.	Max. Conc.	Min. Conc.
0.00	0.01	0.00
0.01	0.03	0.00
0.02	0.05	0.00
0.03	0.08	0.00
0.05	0.15	0.01
0.10	0.30	0.01
0.22	0.66	0.03
0.43	1.31	0.06
0.65	1.97	0.09
0.01	0.03	0.00
0.02	0.05	0.00
0.03	0.10	0.00
0.05	0.15	0.01
0.10	0.30	0.01
0.20	0.61	0.03
0.43	1.31	0.06
0.87	2.62	0.12
1.30	3.93	0.18
0.01	0.04	0.00
0.02	0.08	0.00
0.05	0.15	0.01
0.07	0.23	0.01
0.15	0.45	0.02
0.30	0.90	0.04
0.65	1.96	0.09
1.29	3.91	0.18
1.94	5.87	0.27

Brown Trout (10 - 20 inches)

DDT Tissue Concentration (85 + 86)	ppm
Mean	0.33
Max.	0.62
Min.	0.16
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000003	0.000001
0.114	2	0.000625	0.000003	0.000006	0.000001
0.114	4	0.001249	0.000006	0.000011	0.000003
0.114	6	0.001874	0.000009	0.000017	0.000004
0.114	12	0.003748	0.000018	0.000033	0.000009
0.114	24	0.007496	0.000035	0.000066	0.000017
0.114	52	0.016241	0.000077	0.000144	0.000037
0.114	104	0.032482	0.000153	0.000288	0.000074
0.114	156	0.048723	0.000230	0.000432	0.000111
0.228	1	0.000625	0.000003	0.000006	0.000001
0.228	2	0.001249	0.000006	0.000011	0.000003
0.228	4	0.002499	0.000012	0.000022	0.000006
0.228	6	0.003748	0.000018	0.000033	0.000009
0.228	12	0.007496	0.000035	0.000066	0.000017
0.228	24	0.014992	0.000071	0.000133	0.000034
0.228	52	0.032482	0.000153	0.000288	0.000074
0.228	104	0.064964	0.000306	0.000575	0.000148
0.228	156	0.097447	0.000459	0.000863	0.000223
0.34	1	0.000932	0.000004	0.000008	0.000002
0.34	2	0.001863	0.000009	0.000017	0.000004
0.34	4	0.003726	0.000018	0.000033	0.000009
0.34	6	0.005589	0.000026	0.000050	0.000013
0.34	12	0.011178	0.000053	0.000099	0.000026
0.34	24	0.022356	0.000105	0.000198	0.000051
0.34	52	0.048438	0.000228	0.000429	0.000111
0.34	104	0.096877	0.000457	0.000858	0.000221
0.34	156	0.145315	0.000685	0.001287	0.000332

Brown Trout (10 - 20 inches) continued.

Upper Limit (95%) on Risk q* =.39 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =2.54 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
5.74E-07	1.08E-06	2.78E-07	3.74E-06	7.03E-06	1.81E-06
1.15E-06	2.16E-06	5.57E-07	7.48E-06	1.41E-05	3.63E-06
2.30E-06	4.32E-06	1.11E-06	1.50E-05	2.81E-05	7.25E-06
3.45E-06	6.47E-06	1.67E-06	2.24E-05	4.22E-05	1.09E-05
6.89E-06	1.29E-05	3.34E-06	4.49E-05	8.43E-05	2.18E-05
1.38E-05	2.59E-05	6.68E-06	8.98E-05	1.69E-04	4.35E-05
2.99E-05	5.61E-05	1.45E-05	1.94E-04	3.65E-04	9.43E-05
5.97E-05	1.12E-04	2.90E-05	3.89E-04	7.31E-04	1.89E-04
8.96E-05	1.68E-04	4.34E-05	5.83E-04	1.10E-03	2.83E-04
1.15E-06	2.16E-06	5.57E-07	7.48E-06	1.41E-05	3.63E-06
2.30E-06	4.32E-06	1.11E-06	1.50E-05	2.81E-05	7.25E-06
4.59E-06	8.63E-06	2.23E-06	2.99E-05	5.62E-05	1.45E-05
6.89E-06	1.29E-05	3.34E-06	4.49E-05	8.43E-05	2.18E-05
1.38E-05	2.59E-05	6.68E-06	8.98E-05	1.69E-04	4.35E-05
2.76E-05	5.18E-05	1.34E-05	1.80E-04	3.37E-04	8.70E-05
5.97E-05	1.12E-04	2.90E-05	3.89E-04	7.31E-04	1.89E-04
1.19E-04	2.24E-04	5.79E-05	7.78E-04	1.46E-03	3.77E-04
1.79E-04	3.37E-04	8.69E-05	1.17E-03	2.19E-03	5.66E-04
1.71E-06	3.22E-06	8.30E-07	1.12E-05	2.10E-05	5.41E-06
3.43E-06	6.44E-06	1.66E-06	2.23E-05	4.19E-05	1.08E-05
6.85E-06	1.29E-05	3.32E-06	4.46E-05	8.38E-05	2.16E-05
1.03E-05	1.93E-05	4.98E-06	6.69E-05	1.26E-04	3.24E-05
2.06E-05	3.86E-05	9.96E-06	1.34E-04	2.51E-04	6.49E-05
4.11E-05	7.72E-05	1.99E-05	2.68E-04	5.03E-04	1.30E-04
8.91E-05	1.67E-04	4.32E-05	5.80E-04	1.09E-03	2.81E-04
1.78E-04	3.35E-04	8.64E-05	1.16E-03	2.18E-03	5.62E-04
2.67E-04	5.02E-04	1.30E-04	1.74E-03	3.27E-03	8.44E-04

Brown Trout (10 - 20 inches) continu

Hazard Index
RfD = 0.00046 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.00	0.01	0.00
0.01	0.01	0.00
0.01	0.02	0.01
0.02	0.04	0.01
0.04	0.07	0.02
0.08	0.14	0.04
0.17	0.31	0.08
0.33	0.63	0.16
0.50	0.94	0.24
0.00	0.00	0.00
0.01	0.01	0.00
0.01	0.02	0.01
0.03	0.05	0.01
0.04	0.07	0.02
0.08	0.14	0.04
0.15	0.29	0.07
0.33	0.63	0.16
0.67	1.25	0.32
1.00	1.88	0.48
0.00	0.00	0.00
0.01	0.02	0.00
0.02	0.04	0.01
0.04	0.07	0.02
0.06	0.11	0.03
0.11	0.22	0.06
0.23	0.43	0.11
0.50	0.93	0.24
0.99	1.87	0.48
1.49	2.80	0.72

Brown Trout (20 - 30 inches)

DDT Tissue Concentration (85 + 86)	ppm
Mean	0.53
Max.	1.3
Min.	0.06
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day)		
			Mean	Max.	Min.
0.114	1	0.000312	0.000002	0.000006	0.000000
0.114	2	0.000625	0.000005	0.000012	0.000001
0.114	4	0.001249	0.000009	0.000023	0.000001
0.114	6	0.001874	0.000014	0.000035	0.000002
0.114	12	0.003748	0.000028	0.000070	0.000003
0.114	24	0.007496	0.000057	0.000139	0.000006
0.114	52	0.016241	0.000123	0.000302	0.000014
0.114	104	0.032482	0.000246	0.000603	0.000028
0.114	156	0.048723	0.000369	0.000905	0.000042
0.228	1	0.000625	0.000005	0.000012	0.000001
0.228	2	0.001249	0.000009	0.000023	0.000001
0.228	4	0.002499	0.000019	0.000046	0.000002
0.228	6	0.003748	0.000028	0.000070	0.000003
0.228	12	0.007496	0.000057	0.000139	0.000006
0.228	24	0.014992	0.000114	0.000278	0.000013
0.228	52	0.032482	0.000246	0.000603	0.000028
0.228	104	0.064964	0.000492	0.001206	0.000056
0.228	156	0.097447	0.000738	0.001810	0.000084
0.34	1	0.000932	0.000007	0.000017	0.000001
0.34	2	0.001863	0.000014	0.000035	0.000002
0.34	4	0.003726	0.000028	0.000069	0.000003
0.34	6	0.005589	0.000042	0.000104	0.000005
0.34	12	0.011178	0.000085	0.000208	0.000010
0.34	24	0.022356	0.000169	0.000415	0.000019
0.34	52	0.048438	0.000367	0.000900	0.000042
0.34	104	0.096877	0.000733	0.001799	0.000083
0.34	156	0.145315	0.001100	0.002699	0.000125

Brown Trout (20 - 30 inches continued.

Upper Limit (95%) on Risk q* =.39 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =2.54 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
9.22E-07	2.26E-06	1.04E-07	6.01E-06	1.47E-05	6.80E-07
1.84E-06	4.52E-06	2.09E-07	1.20E-05	2.95E-05	1.36E-06
3.69E-06	9.05E-06	4.18E-07	2.40E-05	5.89E-05	2.72E-06
5.53E-06	1.36E-05	6.26E-07	3.60E-05	8.84E-05	4.08E-06
1.11E-05	2.71E-05	1.25E-06	7.21E-05	1.77E-04	8.16E-06
2.21E-05	5.43E-05	2.51E-06	1.44E-04	3.54E-04	1.63E-05
4.80E-05	1.18E-04	5.43E-06	3.12E-04	7.66E-04	3.54E-05
9.59E-05	2.35E-04	1.09E-05	6.25E-04	1.53E-03	7.07E-05
1.44E-04	3.53E-04	1.63E-05	9.37E-04	2.30E-03	1.06E-04
1.84E-06	4.52E-06	2.09E-07	1.20E-05	2.95E-05	1.36E-06
3.69E-06	9.05E-06	4.18E-07	2.40E-05	5.89E-05	2.72E-06
7.38E-06	1.81E-05	8.35E-07	4.81E-05	1.18E-04	5.44E-06
1.11E-05	2.71E-05	1.25E-06	7.21E-05	1.77E-04	8.16E-06
2.21E-05	5.43E-05	2.51E-06	1.44E-04	3.54E-04	1.63E-05
4.43E-05	1.09E-04	5.01E-06	2.88E-04	7.07E-04	3.26E-05
9.59E-05	2.35E-04	1.09E-05	6.25E-04	1.53E-03	7.07E-05
1.92E-04	4.71E-04	2.17E-05	1.25E-03	3.06E-03	1.41E-04
2.88E-04	7.06E-04	3.26E-05	1.87E-03	4.60E-03	2.12E-04
2.75E-06	6.75E-06	3.11E-07	1.79E-05	4.39E-05	2.03E-06
5.50E-06	1.35E-05	6.23E-07	3.58E-05	8.79E-05	4.06E-06
1.10E-05	2.70E-05	1.25E-06	7.17E-05	1.76E-04	8.11E-06
1.65E-05	4.05E-05	1.87E-06	1.07E-04	2.64E-04	1.22E-05
3.30E-05	8.10E-05	3.74E-06	2.15E-04	5.27E-04	2.43E-05
6.60E-05	1.62E-04	7.47E-06	4.30E-04	1.05E-03	4.87E-05
1.43E-04	3.51E-04	1.62E-05	9.32E-04	2.28E-03	1.05E-04
2.86E-04	7.02E-04	3.24E-05	1.86E-03	4.57E-03	2.11E-04
4.29E-04	1.05E-03	4.86E-05	2.79E-03	6.85E-03	3.16E-04

Brown Trout (20 - 30 inches) continued.

Hazard Index RfD = 0.00046 Liver Mean Conc.	 Max. Conc.	 Min. Conc.
0.01	0.01	0.00
0.01	0.03	0.00
0.02	0.05	0.00
0.03	0.08	0.00
0.06	0.15	0.01
0.12	0.30	0.01
0.27	0.66	0.03
0.53	1.31	0.06
0.80	1.97	0.09
0.00	0.00	0.00
0.01	0.03	0.00
0.02	0.05	0.00
0.04	0.10	0.00
0.06	0.15	0.01
0.12	0.30	0.01
0.25	0.61	0.03
0.53	1.31	0.06
1.07	2.62	0.12
1.60	3.93	0.18
0.00	0.00	0.00
0.02	0.04	0.00
0.03	0.08	0.00
0.06	0.15	0.01
0.09	0.23	0.01
0.18	0.45	0.02
0.37	0.90	0.04
0.80	1.96	0.09
1.59	3.91	0.18
2.39	5.87	0.27

Chinook (All Sizes)

DDT Tissue Concentration (85 + 86)	ppm
Mean	0.74
Max.	4.7
Min.	0
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000003	0.000021	0.000000
0.114	2	0.000625	0.000007	0.000042	0.000000
0.114	4	0.001249	0.000013	0.000084	0.000000
0.114	6	0.001874	0.000020	0.000126	0.000000
0.114	12	0.003748	0.000040	0.000252	0.000000
0.114	24	0.007496	0.000079	0.000503	0.000000
0.114	52	0.016241	0.000172	0.001090	0.000000
0.114	104	0.032482	0.000343	0.002181	0.000000
0.114	156	0.048723	0.000515	0.003271	0.000000
0.228	1	0.000625	0.000007	0.000042	0.000000
0.228	2	0.001249	0.000013	0.000084	0.000000
0.228	4	0.002499	0.000026	0.000168	0.000000
0.228	6	0.003748	0.000040	0.000252	0.000000
0.228	12	0.007496	0.000079	0.000503	0.000000
0.228	24	0.014992	0.000158	0.001007	0.000000
0.228	52	0.032482	0.000343	0.002181	0.000000
0.228	104	0.064964	0.000687	0.004362	0.000000
0.228	156	0.097447	0.001030	0.006543	0.000000
0.34	1	0.000932	0.000010	0.000063	0.000000
0.34	2	0.001863	0.000020	0.000125	0.000000
0.34	4	0.003726	0.000039	0.000250	0.000000
0.34	6	0.005589	0.000059	0.000375	0.000000
0.34	12	0.011178	0.000118	0.000751	0.000000
0.34	24	0.022356	0.000236	0.001501	0.000000
0.34	52	0.048438	0.000512	0.003252	0.000000
0.34	104	0.096877	0.001024	0.006505	0.000000
0.34	156	0.145315	0.001536	0.009757	0.000000

Chinook (All Sizes) continued.

Upper Limit (95%) on Risk q* =.39 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =2.54 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
1.29E-06	8.18E-06	0.00E+00	8.39E-06	5.33E-05	0.00E+00
2.58E-06	1.64E-05	0.00E+00	1.68E-05	1.07E-04	0.00E+00
5.15E-06	3.27E-05	0.00E+00	3.35E-05	2.13E-04	0.00E+00
7.73E-06	4.91E-05	0.00E+00	5.03E-05	3.20E-04	0.00E+00
1.55E-05	9.81E-05	0.00E+00	1.01E-04	6.39E-04	0.00E+00
3.09E-05	1.96E-04	0.00E+00	2.01E-04	1.28E-03	0.00E+00
6.70E-05	4.25E-04	0.00E+00	4.36E-04	2.77E-03	0.00E+00
1.34E-04	8.51E-04	0.00E+00	8.72E-04	5.54E-03	0.00E+00
2.01E-04	1.28E-03	0.00E+00	1.31E-03	8.31E-03	0.00E+00
2.58E-06	1.64E-05	0.00E+00	1.68E-05	1.07E-04	0.00E+00
5.15E-06	3.27E-05	0.00E+00	3.35E-05	2.13E-04	0.00E+00
1.03E-05	6.54E-05	0.00E+00	6.71E-05	4.26E-04	0.00E+00
1.55E-05	9.81E-05	0.00E+00	1.01E-04	6.39E-04	0.00E+00
3.09E-05	1.96E-04	0.00E+00	2.01E-04	1.28E-03	0.00E+00
6.18E-05	3.93E-04	0.00E+00	4.03E-04	2.56E-03	0.00E+00
1.34E-04	8.51E-04	0.00E+00	8.72E-04	5.54E-03	0.00E+00
2.68E-04	1.70E-03	0.00E+00	1.74E-03	1.11E-02	0.00E+00
4.02E-04	2.55E-03	0.00E+00	2.62E-03	1.66E-02	0.00E+00
3.84E-06	2.44E-05	0.00E+00	2.50E-05	1.59E-04	0.00E+00
7.68E-06	4.88E-05	0.00E+00	5.00E-05	3.18E-04	0.00E+00
1.54E-05	9.76E-05	0.00E+00	1.00E-04	6.35E-04	0.00E+00
2.30E-05	1.46E-04	0.00E+00	1.50E-04	9.53E-04	0.00E+00
4.61E-05	2.93E-04	0.00E+00	3.00E-04	1.91E-03	0.00E+00
9.22E-05	5.85E-04	0.00E+00	6.00E-04	3.81E-03	0.00E+00
2.00E-04	1.27E-03	0.00E+00	1.30E-03	8.26E-03	0.00E+00
3.99E-04	2.54E-03	0.00E+00	2.60E-03	1.65E-02	0.00E+00
5.99E-04	3.81E-03	0.00E+00	3.90E-03	2.48E-02	0.00E+00

Chinook (All Sizes) continued.

Hazard Index RfD = 0.00046 Liver Mean Conc.	Max. Conc.	Min. Conc.
0.01	0.05	0.00
0.01	0.09	0.00
0.03	0.18	0.00
0.04	0.27	0.00
0.09	0.55	0.00
0.17	1.09	0.00
0.37	2.37	0.00
0.75	4.74	0.00
1.12	7.11	0.00
0.01	0.09	0.00
0.03	0.18	0.00
0.06	0.36	0.00
0.09	0.55	0.00
0.17	1.09	0.00
0.34	2.19	0.00
0.75	4.74	0.00
1.49	9.48	0.00
2.24	14.22	0.00
0.02	0.14	0.00
0.04	0.27	0.00
0.09	0.54	0.00
0.13	0.82	0.00
0.26	1.63	0.00
0.51	3.26	0.00
1.11	7.07	0.00
2.23	14.14	0.00
3.34	21.21	0.00

Chinook (20 - 30 inches)

DDT Tissue Concentration (85 + 86)	ppm
Mean	0.44
Max.	0.5
Min.	0.38
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000002	0.000002	0.000002
0.114	2	0.000625	0.000004	0.000004	0.000003
0.114	4	0.001249	0.000008	0.000009	0.000007
0.114	6	0.001874	0.000012	0.000013	0.000010
0.114	12	0.003748	0.000024	0.000027	0.000020
0.114	24	0.007496	0.000047	0.000054	0.000041
0.114	52	0.016241	0.000102	0.000116	0.000088
0.114	104	0.032482	0.000204	0.000232	0.000176
0.114	156	0.048723	0.000306	0.000348	0.000264
0.228	1	0.000625	0.000004	0.000004	0.000003
0.228	2	0.001249	0.000008	0.000009	0.000007
0.228	4	0.002499	0.000016	0.000018	0.000014
0.228	6	0.003748	0.000024	0.000027	0.000020
0.228	12	0.007496	0.000047	0.000054	0.000041
0.228	24	0.014992	0.000094	0.000107	0.000081
0.228	52	0.032482	0.000204	0.000232	0.000176
0.228	104	0.064964	0.000408	0.000464	0.000353
0.228	156	0.097447	0.000613	0.000696	0.000529
0.34	1	0.000932	0.000006	0.000007	0.000005
0.34	2	0.001863	0.000012	0.000013	0.000010
0.34	4	0.003726	0.000023	0.000027	0.000020
0.34	6	0.005589	0.000035	0.000040	0.000030
0.34	12	0.011178	0.000070	0.000080	0.000061
0.34	24	0.022356	0.000141	0.000160	0.000121
0.34	52	0.048438	0.000304	0.000346	0.000263
0.34	104	0.096877	0.000609	0.000692	0.000526
0.34	156	0.145315	0.000913	0.001038	0.000789

Chinook (20 - 30 inches) continued.

Upper Limit (95%) on Risk q* =.39 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =2.54 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
7.66E-07	8.70E-07	6.61E-07	4.99E-06	5.67E-06	4.31E-06
1.53E-06	1.74E-06	1.32E-06	9.97E-06	1.13E-05	8.61E-06
3.06E-06	3.48E-06	2.64E-06	1.99E-05	2.27E-05	1.72E-05
4.59E-06	5.22E-06	3.97E-06	2.99E-05	3.40E-05	2.58E-05
9.19E-06	1.04E-05	7.93E-06	5.98E-05	6.80E-05	5.17E-05
1.84E-05	2.09E-05	1.59E-05	1.20E-04	1.36E-04	1.03E-04
3.98E-05	4.52E-05	3.44E-05	2.59E-04	2.95E-04	2.24E-04
7.96E-05	9.05E-05	6.88E-05	5.19E-04	5.89E-04	4.48E-04
1.19E-04	1.36E-04	1.03E-04	7.78E-04	8.84E-04	6.72E-04
1.53E-06	1.74E-06	1.32E-06	9.97E-06	1.13E-05	8.61E-06
3.06E-06	3.48E-06	2.64E-06	1.99E-05	2.27E-05	1.72E-05
6.13E-06	6.96E-06	5.29E-06	3.99E-05	4.53E-05	3.45E-05
9.19E-06	1.04E-05	7.93E-06	5.98E-05	6.80E-05	5.17E-05
1.84E-05	2.09E-05	1.59E-05	1.20E-04	1.36E-04	1.03E-04
3.68E-05	4.18E-05	3.17E-05	2.39E-04	2.72E-04	2.07E-04
7.96E-05	9.05E-05	6.88E-05	5.19E-04	5.89E-04	4.48E-04
1.59E-04	1.81E-04	1.38E-04	1.04E-03	1.18E-03	8.96E-04
2.39E-04	2.71E-04	2.06E-04	1.56E-03	1.77E-03	1.34E-03
2.28E-06	2.59E-06	1.97E-06	1.49E-05	1.69E-05	1.28E-05
4.57E-06	5.19E-06	3.94E-06	2.97E-05	3.38E-05	2.57E-05
9.13E-06	1.04E-05	7.89E-06	5.95E-05	6.76E-05	5.14E-05
1.37E-05	1.56E-05	1.18E-05	8.92E-05	1.01E-04	7.71E-05
2.74E-05	3.11E-05	2.37E-05	1.78E-04	2.03E-04	1.54E-04
5.48E-05	6.23E-05	4.73E-05	3.57E-04	4.06E-04	3.08E-04
1.19E-04	1.35E-04	1.03E-04	7.73E-04	8.79E-04	6.68E-04
2.37E-04	2.70E-04	2.05E-04	1.55E-03	1.76E-03	1.34E-03
3.56E-04	4.05E-04	3.08E-04	2.32E-03	2.64E-03	2.00E-03

Chinook (20 - 30 inches) continued.

Hazard Index
RfD = 0.00046 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.00	0.00	0.00
0.01	0.01	0.01
0.02	0.02	0.01
0.03	0.03	0.02
0.05	0.06	0.04
0.10	0.12	0.09
0.22	0.25	0.19
0.44	0.50	0.38
0.67	0.76	0.57
0.01	0.01	0.01
0.02	0.02	0.01
0.03	0.04	0.03
0.05	0.06	0.04
0.10	0.12	0.09
0.20	0.23	0.18
0.44	0.50	0.38
0.89	1.01	0.77
1.33	1.51	1.15
0.01	0.01	0.01
0.03	0.03	0.02
0.05	0.06	0.04
0.08	0.09	0.07
0.15	0.17	0.13
0.31	0.35	0.26
0.66	0.75	0.57
1.32	1.50	1.14
1.99	2.26	1.71

Chinook (> 30 inches)

DDT Tissue Concentration (85 + 86)	ppm
Mean	1.78
Max.	4.7
Min.	0.66
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000008	0.000021	0.000003
0.114	2	0.000625	0.000016	0.000042	0.000006
0.114	4	0.001249	0.000032	0.000084	0.000012
0.114	6	0.001874	0.000048	0.000126	0.000018
0.114	12	0.003748	0.000095	0.000252	0.000035
0.114	24	0.007496	0.000191	0.000503	0.000071
0.114	52	0.016241	0.000413	0.001090	0.000153
0.114	104	0.032482	0.000826	0.002181	0.000306
0.114	156	0.048723	0.001239	0.003271	0.000459
0.228	1	0.000625	0.000016	0.000042	0.000006
0.228	2	0.001249	0.000032	0.000084	0.000012
0.228	4	0.002499	0.000064	0.000168	0.000024
0.228	6	0.003748	0.000095	0.000252	0.000035
0.228	12	0.007496	0.000191	0.000503	0.000071
0.228	24	0.014992	0.000381	0.001007	0.000141
0.228	52	0.032482	0.000826	0.002181	0.000306
0.228	104	0.064964	0.001652	0.004362	0.000613
0.228	156	0.097447	0.002478	0.006543	0.000919
0.34	1	0.000932	0.000024	0.000063	0.000009
0.34	2	0.001863	0.000047	0.000125	0.000018
0.34	4	0.003726	0.000095	0.000250	0.000035
0.34	6	0.005589	0.000142	0.000375	0.000053
0.34	12	0.011178	0.000284	0.000751	0.000105
0.34	24	0.022356	0.000568	0.001501	0.000211
0.34	52	0.048438	0.001232	0.003252	0.000457
0.34	104	0.096877	0.002463	0.006505	0.000913
0.34	156	0.145315	0.003695	0.009757	0.001370

Chinook (> 30 inches) continued.

Upper Limit (95%) on Risk q* =.39 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =2.54 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
3.10E-06	8.18E-06	1.15E-06	2.02E-05	5.33E-05	7.48E-06
6.19E-06	1.64E-05	2.30E-06	4.03E-05	1.07E-04	1.50E-05
1.24E-05	3.27E-05	4.59E-06	8.07E-05	2.13E-04	2.99E-05
1.86E-05	4.91E-05	6.89E-06	1.21E-04	3.20E-04	4.49E-05
3.72E-05	9.81E-05	1.38E-05	2.42E-04	6.39E-04	8.98E-05
7.43E-05	1.96E-04	2.76E-05	4.84E-04	1.28E-03	1.80E-04
1.61E-04	4.25E-04	5.97E-05	1.05E-03	2.77E-03	3.89E-04
3.22E-04	8.51E-04	1.19E-04	2.10E-03	5.54E-03	7.78E-04
4.83E-04	1.28E-03	1.79E-04	3.15E-03	8.31E-03	1.17E-03
6.19E-06	1.64E-05	2.30E-06	4.03E-05	1.07E-04	1.50E-05
1.24E-05	3.27E-05	4.59E-06	8.07E-05	2.13E-04	2.99E-05
2.48E-05	6.54E-05	9.19E-06	1.61E-04	4.26E-04	5.98E-05
3.72E-05	9.81E-05	1.38E-05	2.42E-04	6.39E-04	8.98E-05
7.43E-05	1.96E-04	2.76E-05	4.84E-04	1.28E-03	1.80E-04
1.49E-04	3.93E-04	5.51E-05	9.68E-04	2.56E-03	3.59E-04
3.22E-04	8.51E-04	1.19E-04	2.10E-03	5.54E-03	7.78E-04
6.44E-04	1.70E-03	2.39E-04	4.20E-03	1.11E-02	1.56E-03
9.66E-04	2.55E-03	3.58E-04	6.29E-03	1.66E-02	2.33E-03
9.24E-06	2.44E-05	3.43E-06	6.02E-05	1.59E-04	2.23E-05
1.85E-05	4.88E-05	6.85E-06	1.20E-04	3.18E-04	4.46E-05
3.70E-05	9.76E-05	1.37E-05	2.41E-04	6.35E-04	8.92E-05
5.54E-05	1.46E-04	2.06E-05	3.61E-04	9.53E-04	1.34E-04
1.11E-04	2.93E-04	4.11E-05	7.22E-04	1.91E-03	2.68E-04
2.22E-04	5.85E-04	8.22E-05	1.44E-03	3.81E-03	5.35E-04
4.80E-04	1.27E-03	1.78E-04	3.13E-03	8.26E-03	1.16E-03
9.61E-04	2.54E-03	3.56E-04	6.26E-03	1.65E-02	2.32E-03
1.44E-03	3.81E-03	5.34E-04	9.39E-03	2.48E-02	3.48E-03

Chinook (> 30 inches) continued.

Hazard Index RfD = 0.00046 Liver Mean Conc.	Max. Conc.	Min. Conc.
0.02	0.05	0.01
0.03	0.09	0.01
0.07	0.18	0.03
0.10	0.27	0.04
0.21	0.55	0.08
0.41	1.09	0.15
0.90	2.37	0.33
1.80	4.74	0.67
2.69	7.11	1.00
0.03	0.09	0.01
0.07	0.18	0.03
0.14	0.36	0.05
0.21	0.55	0.08
0.41	1.09	0.15
0.83	2.19	0.31
1.80	4.74	0.67
3.59	9.48	1.33
5.39	14.22	2.00
0.05	0.14	0.02
0.10	0.27	0.04
0.21	0.54	0.08
0.31	0.82	0.11
0.62	1.63	0.23
1.24	3.26	0.46
2.68	7.07	0.99
5.36	14.14	1.99
8.03	21.21	2.98

Coho (All Sizes)

DDT Tissue Concentration (85 + 86)	ppm
Mean	0.19
Max.	0.69
Min.	0.02
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day)		
			Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000003	0.000000
0.114	2	0.000625	0.000002	0.000006	0.000000
0.114	4	0.001249	0.000003	0.000012	0.000000
0.114	6	0.001874	0.000005	0.000018	0.000001
0.114	12	0.003748	0.000010	0.000037	0.000001
0.114	24	0.007496	0.000020	0.000074	0.000002
0.114	52	0.016241	0.000044	0.000160	0.000005
0.114	104	0.032482	0.000088	0.000320	0.000009
0.114	156	0.048723	0.000132	0.000480	0.000014
0.228	1	0.000625	0.000002	0.000006	0.000000
0.228	2	0.001249	0.000003	0.000012	0.000000
0.228	4	0.002499	0.000007	0.000025	0.000001
0.228	6	0.003748	0.000010	0.000037	0.000001
0.228	12	0.007496	0.000020	0.000074	0.000002
0.228	24	0.014992	0.000041	0.000148	0.000004
0.228	52	0.032482	0.000088	0.000320	0.000009
0.228	104	0.064964	0.000176	0.000640	0.000019
0.228	156	0.097447	0.000264	0.000961	0.000028
0.34	1	0.000932	0.000003	0.000009	0.000000
0.34	2	0.001863	0.000005	0.000018	0.000001
0.34	4	0.003726	0.000010	0.000037	0.000001
0.34	6	0.005589	0.000015	0.000055	0.000002
0.34	12	0.011178	0.000030	0.000110	0.000003
0.34	24	0.022356	0.000061	0.000220	0.000006
0.34	52	0.048438	0.000131	0.000477	0.000014
0.34	104	0.096877	0.000263	0.000955	0.000028
0.34	156	0.145315	0.000394	0.001432	0.000042

Coho (All Sizes) continued.

Upper Limit (95%) on Risk			Upper Limit (95%) on Risk		
q* =.39 Mg/Kg/Day-1			q* =2.54 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
3.31E-07	1.20E-06	3.48E-08	2.15E-06	7.82E-06	2.27E-07
6.61E-07	2.40E-06	6.96E-08	4.31E-06	1.56E-05	4.53E-07
1.32E-06	4.80E-06	1.39E-07	8.61E-06	3.13E-05	9.07E-07
1.98E-06	7.20E-06	2.09E-07	1.29E-05	4.69E-05	1.36E-06
3.97E-06	1.44E-05	4.18E-07	2.58E-05	9.38E-05	2.72E-06
7.93E-06	2.88E-05	8.35E-07	5.17E-05	1.88E-04	5.44E-06
1.72E-05	6.24E-05	1.81E-06	1.12E-04	4.07E-04	1.18E-05
3.44E-05	1.25E-04	3.62E-06	2.24E-04	8.13E-04	2.36E-05
5.16E-05	1.87E-04	5.43E-06	3.36E-04	1.22E-03	3.54E-05
6.61E-07	2.40E-06	6.96E-08	4.31E-06	1.56E-05	4.53E-07
1.32E-06	4.80E-06	1.39E-07	8.61E-06	3.13E-05	9.07E-07
2.64E-06	9.61E-06	2.78E-07	1.72E-05	6.26E-05	1.81E-06
3.97E-06	1.44E-05	4.18E-07	2.58E-05	9.38E-05	2.72E-06
7.93E-06	2.88E-05	8.35E-07	5.17E-05	1.88E-04	5.44E-06
1.59E-05	5.76E-05	1.67E-06	1.03E-04	3.75E-04	1.09E-05
3.44E-05	1.25E-04	3.62E-06	2.24E-04	8.13E-04	2.36E-05
6.88E-05	2.50E-04	7.24E-06	4.48E-04	1.63E-03	4.71E-05
1.03E-04	3.75E-04	1.09E-05	6.72E-04	2.44E-03	7.07E-05
9.86E-07	3.58E-06	1.04E-07	6.42E-06	2.33E-05	6.76E-07
1.97E-06	7.16E-06	2.08E-07	1.28E-05	4.66E-05	1.35E-06
3.94E-06	1.43E-05	4.15E-07	2.57E-05	9.33E-05	2.70E-06
5.92E-06	2.15E-05	6.23E-07	3.85E-05	1.40E-04	4.06E-06
1.18E-05	4.30E-05	1.25E-06	7.71E-05	2.80E-04	8.11E-06
2.37E-05	8.59E-05	2.49E-06	1.54E-04	5.60E-04	1.62E-05
5.13E-05	1.86E-04	5.40E-06	3.34E-04	1.21E-03	3.52E-05
1.03E-04	3.72E-04	1.08E-05	6.68E-04	2.43E-03	7.03E-05
1.54E-04	5.59E-04	1.62E-05	1.00E-03	3.64E-03	1.05E-04

Coho (All Sizes) continued.

Hazard Index RfD =	 0.00046 Liver	
Mean Conc.	Max. Conc.	Min. Conc.
0.00	0.01	0.00
0.00	0.01	0.00
0.01	0.03	0.00
0.01	0.04	0.00
0.02	0.08	0.00
0.04	0.16	0.00
0.10	0.35	0.01
0.19	0.70	0.02
0.29	1.04	0.03
0.00	0.01	0.00
0.01	0.03	0.00
0.01	0.05	0.00
0.02	0.08	0.00
0.04	0.16	0.00
0.09	0.32	0.01
0.19	0.70	0.02
0.38	1.39	0.04
0.57	2.09	0.06
0.01	0.02	0.00
0.01	0.04	0.00
0.02	0.08	0.00
0.03	0.12	0.00
0.07	0.24	0.01
0.13	0.48	0.01
0.29	1.04	0.03
0.57	2.08	0.06
0.86	3.11	0.09

Lake Trout (All Sizes)

DDT Tissue Concentration (85 + 86)	ppm
Mean	0.9
Max.	3.1
Min.	0.12
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000004	0.000014	0.000001
0.114	2	0.000625	0.000008	0.000028	0.000001
0.114	4	0.001249	0.000016	0.000055	0.000002
0.114	6	0.001874	0.000024	0.000083	0.000003
0.114	12	0.003748	0.000048	0.000166	0.000006
0.114	24	0.007496	0.000096	0.000332	0.000013
0.114	52	0.016241	0.000209	0.000719	0.000028
0.114	104	0.032482	0.000418	0.001438	0.000056
0.114	156	0.048723	0.000626	0.002158	0.000084
0.228	1	0.000625	0.000008	0.000028	0.000001
0.228	2	0.001249	0.000016	0.000055	0.000002
0.228	4	0.002499	0.000032	0.000111	0.000004
0.228	6	0.003748	0.000048	0.000166	0.000006
0.228	12	0.007496	0.000096	0.000332	0.000013
0.228	24	0.014992	0.000193	0.000664	0.000026
0.228	52	0.032482	0.000418	0.001438	0.000056
0.228	104	0.064964	0.000835	0.002877	0.000111
0.228	156	0.097447	0.001253	0.004315	0.000167
0.34	1	0.000932	0.000012	0.000041	0.000002
0.34	2	0.001863	0.000024	0.000083	0.000003
0.34	4	0.003726	0.000048	0.000165	0.000006
0.34	6	0.005589	0.000072	0.000248	0.000010
0.34	12	0.011178	0.000144	0.000495	0.000019
0.34	24	0.022356	0.000287	0.000990	0.000038
0.34	52	0.048438	0.000623	0.002145	0.000083
0.34	104	0.096877	0.001246	0.004290	0.000166
0.34	156	0.145315	0.001868	0.006435	0.000249

Lake Trout (All Sizes) continued.

Upper Limit (95%) on Risk q* =.39 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =2.54 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
1.57E-06	5.39E-06	2.09E-07	1.02E-05	3.51E-05	1.36E-06
3.13E-06	1.08E-05	4.18E-07	2.04E-05	7.03E-05	2.72E-06
6.26E-06	2.16E-05	8.35E-07	4.08E-05	1.41E-04	5.44E-06
9.40E-06	3.24E-05	1.25E-06	6.12E-05	2.11E-04	8.16E-06
1.88E-05	6.47E-05	2.51E-06	1.22E-04	4.22E-04	1.63E-05
3.76E-05	1.29E-04	5.01E-06	2.45E-04	8.43E-04	3.26E-05
8.14E-05	2.81E-04	1.09E-05	5.30E-04	1.83E-03	7.07E-05
1.63E-04	5.61E-04	2.17E-05	1.06E-03	3.65E-03	1.41E-04
2.44E-04	8.42E-04	3.26E-05	1.59E-03	5.48E-03	2.12E-04
3.13E-06	1.08E-05	4.18E-07	2.04E-05	7.03E-05	2.72E-06
6.26E-06	2.16E-05	8.35E-07	4.08E-05	1.41E-04	5.44E-06
1.25E-05	4.32E-05	1.67E-06	8.16E-05	2.81E-04	1.09E-05
1.88E-05	6.47E-05	2.51E-06	1.22E-04	4.22E-04	1.63E-05
3.76E-05	1.29E-04	5.01E-06	2.45E-04	8.43E-04	3.26E-05
7.52E-05	2.59E-04	1.00E-05	4.90E-04	1.69E-03	6.53E-05
1.63E-04	5.61E-04	2.17E-05	1.06E-03	3.65E-03	1.41E-04
3.26E-04	1.12E-03	4.34E-05	2.12E-03	7.31E-03	2.83E-04
4.89E-04	1.68E-03	6.51E-05	3.18E-03	1.10E-02	4.24E-04
4.67E-06	1.61E-05	6.23E-07	3.04E-05	1.05E-04	4.06E-06
9.34E-06	3.22E-05	1.25E-06	6.08E-05	2.10E-04	8.11E-06
1.87E-05	6.44E-05	2.49E-06	1.22E-04	4.19E-04	1.62E-05
2.80E-05	9.65E-05	3.74E-06	1.83E-04	6.29E-04	2.43E-05
5.61E-05	1.93E-04	7.47E-06	3.65E-04	1.26E-03	4.87E-05
1.12E-04	3.86E-04	1.49E-05	7.30E-04	2.51E-03	9.73E-05
2.43E-04	8.37E-04	3.24E-05	1.58E-03	5.45E-03	2.11E-04
4.86E-04	1.67E-03	6.48E-05	3.16E-03	1.09E-02	4.22E-04
7.29E-04	2.51E-03	9.72E-05	4.75E-03	1.63E-02	6.33E-04

Lake Trout (All Sizes) continued.

Hazard Index RfD = 0.00046 Liver Mean Conc.	Max. Conc.	Min. Conc.
0.01	0.03	0.00
0.02	0.06	0.00
0.03	0.12	0.00
0.05	0.18	0.01
0.10	0.36	0.01
0.21	0.72	0.03
0.45	1.56	0.06
0.91	3.13	0.12
1.36	4.69	0.18
0.02	0.06	0.00
0.03	0.12	0.00
0.07	0.24	0.01
0.10	0.36	0.01
0.21	0.72	0.03
0.42	1.44	0.06
0.91	3.13	0.12
1.82	6.25	0.24
2.72	9.38	0.36
0.03	0.09	0.00
0.05	0.18	0.01
0.10	0.36	0.01
0.16	0.54	0.02
0.31	1.08	0.04
0.62	2.15	0.08
1.35	4.66	0.18
2.71	9.33	0.36
4.06	13.99	0.54

Lake Trout (10 - 20 inches)

DDT Tissue Concentration (85 + 86)	ppm
Mean	0.23
Max.	
Min.	
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000001		
0.114	2	0.000625	0.000002		
0.114	4	0.001249	0.000004		
0.114	6	0.001874	0.000006		
0.114	12	0.003748	0.000012		
0.114	24	0.007496	0.000025		
0.114	52	0.016241	0.000053		
0.114	104	0.032482	0.000107		
0.114	156	0.048723	0.000160		
0.228	1	0.000625	0.000002		
0.228	2	0.001249	0.000004		
0.228	4	0.002499	0.000008		
0.228	6	0.003748	0.000012		
0.228	12	0.007496	0.000025		
0.228	24	0.014992	0.000049		
0.228	52	0.032482	0.000107		
0.228	104	0.064964	0.000213		
0.228	156	0.097447	0.000320		
0.34	1	0.000932	0.000003		
0.34	2	0.001863	0.000006		
0.34	4	0.003726	0.000012		
0.34	6	0.005589	0.000018		
0.34	12	0.011178	0.000037		
0.34	24	0.022356	0.000073		
0.34	52	0.048438	0.000159		
0.34	104	0.096877	0.000318		
0.34	156	0.145315	0.000477		

Lake Trout (10 - 20 inches) continued.

Upper Limit (95%) on Risk q* =.39 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =2.54 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
4.00E-07			2.61E-06		
8.00E-07			5.21E-06		
1.60E-06			1.04E-05		
2.40E-06			1.56E-05		
4.80E-06			3.13E-05		
9.61E-06			6.26E-05		
2.08E-05			1.36E-04		
4.16E-05			2.71E-04		
6.24E-05			4.07E-04		
8.00E-07			5.21E-06		
1.60E-06			1.04E-05		
3.20E-06			2.09E-05		
4.80E-06			3.13E-05		
9.61E-06			6.26E-05		
1.92E-05			1.25E-04		
4.16E-05			2.71E-04		
8.32E-05			5.42E-04		
1.25E-04			8.13E-04		
1.19E-06			7.77E-06		
2.39E-06			1.55E-05		
4.77E-06			3.11E-05		
7.16E-06			4.66E-05		
1.43E-05			9.33E-05		
2.86E-05			1.87E-04		
6.21E-05			4.04E-04		
1.24E-04			8.09E-04		
1.86E-04			1.21E-03		

Lake Trout (10 - 20 inches) continued.

Hazard Index		
RfD =	0.00046 Liver	
Mean Conc.	Max. Conc.	Min. Conc.
0.00		
0.00		
0.01		
0.01		
0.03		
0.05		
0.12		
0.23		
0.35		
0.00		
0.00		
0.01		
0.02		
0.03		
0.05		
0.11		
0.23		
0.46		
0.70		
0.00		
0.01		
0.01		
0.03		
0.04		
0.08		
0.16		
0.35		
0.69		
1.04		

Lake Trout (20 - 30 inches)

DDT Tissue Contration (85 + 86)	ppm
Mean	1.2
Max.	3.1
Min.	0.7
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000005	0.000014	0.000003
0.114	2	0.000625	0.000011	0.000028	0.000006
0.114	4	0.001249	0.000021	0.000055	0.000012
0.114	6	0.001874	0.000032	0.000083	0.000019
0.114	12	0.003748	0.000064	0.000166	0.000037
0.114	24	0.007496	0.000129	0.000332	0.000075
0.114	52	0.016241	0.000278	0.000719	0.000162
0.114	104	0.032482	0.000557	0.001438	0.000325
0.114	156	0.048723	0.000835	0.002158	0.000487
0.228	1	0.000625	0.000011	0.000028	0.000006
0.228	2	0.001249	0.000021	0.000055	0.000012
0.228	4	0.002499	0.000043	0.000111	0.000025
0.228	6	0.003748	0.000064	0.000166	0.000037
0.228	12	0.007496	0.000129	0.000332	0.000075
0.228	24	0.014992	0.000257	0.000664	0.000150
0.228	52	0.032482	0.000557	0.001438	0.000325
0.228	104	0.064964	0.001114	0.002877	0.000650
0.228	156	0.097447	0.001671	0.004315	0.000974
0.34	1	0.000932	0.000016	0.000041	0.000009
0.34	2	0.001863	0.000032	0.000083	0.000019
0.34	4	0.003726	0.000064	0.000165	0.000037
0.34	6	0.005589	0.000096	0.000248	0.000056
0.34	12	0.011178	0.000192	0.000495	0.000112
0.34	24	0.022356	0.000383	0.000990	0.000224
0.34	52	0.048438	0.000830	0.002145	0.000484
0.34	104	0.096877	0.001661	0.004290	0.000969
0.34	156	0.145315	0.002491	0.006435	0.001453

Lake Trout (20 - 30 inches) continued.

Upper Limit (95%) on Risk q* =.39 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =2.54 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
2.09E-06	5.39E-06	1.22E-06	1.36E-05	3.51E-05	7.93E-06
4.18E-06	1.08E-05	2.44E-06	2.72E-05	7.03E-05	1.59E-05
8.35E-06	2.16E-05	4.87E-06	5.44E-05	1.41E-04	3.17E-05
1.25E-05	3.24E-05	7.31E-06	8.16E-05	2.11E-04	4.76E-05
2.51E-05	6.47E-05	1.46E-05	1.63E-04	4.22E-04	9.52E-05
5.01E-05	1.29E-04	2.92E-05	3.26E-04	8.43E-04	1.90E-04
1.09E-04	2.81E-04	6.33E-05	7.07E-04	1.83E-03	4.13E-04
2.17E-04	5.61E-04	1.27E-04	1.41E-03	3.65E-03	8.25E-04
3.26E-04	8.42E-04	1.90E-04	2.12E-03	5.48E-03	1.24E-03
4.18E-06	1.08E-05	2.44E-06	2.72E-05	7.03E-05	1.59E-05
8.35E-06	2.16E-05	4.87E-06	5.44E-05	1.41E-04	3.17E-05
1.67E-05	4.32E-05	9.74E-06	1.09E-04	2.81E-04	6.35E-05
2.51E-05	6.47E-05	1.46E-05	1.63E-04	4.22E-04	9.52E-05
5.01E-05	1.29E-04	2.92E-05	3.26E-04	8.43E-04	1.90E-04
1.00E-04	2.59E-04	5.85E-05	6.53E-04	1.69E-03	3.81E-04
2.17E-04	5.61E-04	1.27E-04	1.41E-03	3.65E-03	8.25E-04
4.34E-04	1.12E-03	2.53E-04	2.83E-03	7.31E-03	1.65E-03
6.51E-04	1.68E-03	3.80E-04	4.24E-03	1.10E-02	2.48E-03
6.23E-06	1.61E-05	3.63E-06	4.06E-05	1.05E-04	2.37E-05
1.25E-05	3.22E-05	7.27E-06	8.11E-05	2.10E-04	4.73E-05
2.49E-05	6.44E-05	1.45E-05	1.62E-04	4.19E-04	9.46E-05
3.74E-05	9.65E-05	2.18E-05	2.43E-04	6.29E-04	1.42E-04
7.47E-05	1.93E-04	4.36E-05	4.87E-04	1.26E-03	2.84E-04
1.49E-04	3.86E-04	8.72E-05	9.73E-04	2.51E-03	5.68E-04
3.24E-04	8.37E-04	1.89E-04	2.11E-03	5.45E-03	1.23E-03
6.48E-04	1.67E-03	3.78E-04	4.22E-03	1.09E-02	2.46E-03
9.72E-04	2.51E-03	5.67E-04	6.33E-03	1.63E-02	3.69E-03

Lake Trout (20 - 30 inches) continued.

	Hazard Index	
RfD =	0.00046 Liver	
Mean Conc.	Max. Conc.	Min. Conc.
0.01	0.03	0.01
0.02	0.06	0.01
0.05	0.12	0.03
0.07	0.18	0.04
0.14	0.36	0.08
0.28	0.72	0.16
0.61	1.56	0.35
1.21	3.13	0.71
1.82	4.69	1.06
0.02	0.06	0.01
0.05	0.12	0.03
0.09	0.24	0.05
0.14	0.36	0.08
0.28	0.72	0.16
0.56	1.44	0.33
1.21	3.13	0.71
2.42	6.25	1.41
3.63	9.38	2.12
0.03	0.09	0.02
0.07	0.18	0.04
0.14	0.36	0.08
0.21	0.54	0.12
0.42	1.08	0.24
0.83	2.15	0.49
1.81	4.66	1.05
3.61	9.33	2.11
5.42	13.99	3.16

Perch (All Sizes)

DDT Tissue Concentration (85 + 86)	ppm
Mean	0.06
Max.	0.13
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day)		
			Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000001	0.000000
0.114	2	0.000625	0.000001	0.000001	0.000000
0.114	4	0.001249	0.000001	0.000002	0.000000
0.114	6	0.001874	0.000002	0.000003	0.000000
0.114	12	0.003748	0.000003	0.000007	0.000000
0.114	24	0.007496	0.000006	0.000014	0.000000
0.114	52	0.016241	0.000014	0.000030	0.000000
0.114	104	0.032482	0.000028	0.000060	0.000000
0.114	156	0.048723	0.000042	0.000090	0.000000
			0.000000	0.000000	0.000000
0.228	1	0.000625	0.000001	0.000001	0.000000
0.228	2	0.001249	0.000001	0.000002	0.000000
0.228	4	0.002499	0.000002	0.000005	0.000000
0.228	6	0.003748	0.000003	0.000007	0.000000
0.228	12	0.007496	0.000006	0.000014	0.000000
0.228	24	0.014992	0.000013	0.000028	0.000000
0.228	52	0.032482	0.000028	0.000060	0.000000
0.228	104	0.064964	0.000056	0.000121	0.000000
0.228	156	0.097447	0.000084	0.000181	0.000000
			0.000000	0.000000	0.000000
0.34	1	0.000932	0.000001	0.000002	0.000000
0.34	2	0.001863	0.000002	0.000003	0.000000
0.34	4	0.003726	0.000003	0.000007	0.000000
0.34	6	0.005589	0.000005	0.000010	0.000000
0.34	12	0.011178	0.000010	0.000021	0.000000
0.34	24	0.022356	0.000019	0.000042	0.000000
0.34	52	0.048438	0.000042	0.000090	0.000000
0.34	104	0.096877	0.000083	0.000180	0.000000
0.34	156	0.145315	0.000125	0.000270	0.000000

Perch (All Sizes) continued.

Upper Limit (95%) on Risk q* =.39 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =2.54 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
1.04E-07	2.26E-07	0.00E+00	6.80E-07	1.47E-06	0.00E+00
2.09E-07	4.52E-07	0.00E+00	1.36E-06	2.95E-06	0.00E+00
4.18E-07	9.05E-07	0.00E+00	2.72E-06	5.89E-06	0.00E+00
6.26E-07	1.36E-06	0.00E+00	4.08E-06	8.84E-06	0.00E+00
1.25E-06	2.71E-06	0.00E+00	8.16E-06	1.77E-05	0.00E+00
2.51E-06	5.43E-06	0.00E+00	1.63E-05	3.54E-05	0.00E+00
5.43E-06	1.18E-05	0.00E+00	3.54E-05	7.66E-05	0.00E+00
1.09E-05	2.35E-05	0.00E+00	7.07E-05	1.53E-04	0.00E+00
1.63E-05	3.53E-05	0.00E+00	1.06E-04	2.30E-04	0.00E+00
2.09E-07	4.52E-07	0.00E+00	1.36E-06	2.95E-06	0.00E+00
4.18E-07	9.05E-07	0.00E+00	2.72E-06	5.89E-06	0.00E+00
8.35E-07	1.81E-06	0.00E+00	5.44E-06	1.18E-05	0.00E+00
1.25E-06	2.71E-06	0.00E+00	8.16E-06	1.77E-05	0.00E+00
2.51E-06	5.43E-06	0.00E+00	1.63E-05	3.54E-05	0.00E+00
5.01E-06	1.09E-05	0.00E+00	3.26E-05	7.07E-05	0.00E+00
1.09E-05	2.35E-05	0.00E+00	7.07E-05	1.53E-04	0.00E+00
2.17E-05	4.71E-05	0.00E+00	1.41E-04	3.06E-04	0.00E+00
3.26E-05	7.06E-05	0.00E+00	2.12E-04	4.60E-04	0.00E+00
3.11E-07	6.75E-07	0.00E+00	2.03E-06	4.39E-06	0.00E+00
6.23E-07	1.35E-06	0.00E+00	4.06E-06	8.79E-06	0.00E+00
1.25E-06	2.70E-06	0.00E+00	8.11E-06	1.76E-05	0.00E+00
1.87E-06	4.05E-06	0.00E+00	1.22E-05	2.64E-05	0.00E+00
3.74E-06	8.10E-06	0.00E+00	2.43E-05	5.27E-05	0.00E+00
7.47E-06	1.62E-05	0.00E+00	4.87E-05	1.05E-04	0.00E+00
1.62E-05	3.51E-05	0.00E+00	1.05E-04	2.28E-04	0.00E+00
3.24E-05	7.02E-05	0.00E+00	2.11E-04	4.57E-04	0.00E+00
4.86E-05	1.05E-04	0.00E+00	3.16E-04	6.85E-04	0.00E+00

Perch (All Sizes) continued.

	Hazard Index	
RfD =	0.00046 Liver	
Mean Conc.	Max. Conc.	Min. Conc.
0.00	0.00	0.00
0.00	0.00	0.00
0.00	0.01	0.00
0.00	0.01	0.00
0.01	0.02	0.00
0.01	0.03	0.00
0.03	0.07	0.00
0.06	0.13	0.00
0.09	0.20	0.00
0.00	0.00	0.00
0.00	0.01	0.00
0.00	0.01	0.00
0.01	0.02	0.00
0.01	0.03	0.00
0.03	0.06	0.00
0.06	0.13	0.00
0.12	0.26	0.00
0.18	0.39	0.00
0.00	0.00	0.00
0.00	0.01	0.00
0.01	0.02	0.00
0.01	0.02	0.00
0.02	0.05	0.00
0.04	0.09	0.00
0.09	0.20	0.00
0.18	0.39	0.00
0.27	0.59	0.00

Walleye (All Sizes)

DDT Tissue Concentration (85 + 86)	ppm
Mean	0.11
Max.	0.47
Min.	0.03

Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000002	0.000000
0.114	2	0.000625	0.000001	0.000004	0.000000
0.114	4	0.001249	0.000002	0.000008	0.000001
0.114	6	0.001874	0.000003	0.000013	0.000001
0.114	12	0.003748	0.000006	0.000025	0.000002
0.114	24	0.007496	0.000012	0.000050	0.000003
0.114	52	0.016241	0.000026	0.000109	0.000007
0.114	104	0.032482	0.000051	0.000218	0.000014
0.114	156	0.048723	0.000077	0.000327	0.000021
0.228	1	0.000625	0.000001	0.000004	0.000000
0.228	2	0.001249	0.000002	0.000008	0.000001
0.228	4	0.002499	0.000004	0.000017	0.000001
0.228	6	0.003748	0.000006	0.000025	0.000002
0.228	12	0.007496	0.000012	0.000050	0.000003
0.228	24	0.014992	0.000024	0.000101	0.000006
0.228	52	0.032482	0.000051	0.000218	0.000014
0.228	104	0.064964	0.000102	0.000436	0.000028
0.228	156	0.097447	0.000153	0.000654	0.000042
0.34	1	0.000932	0.000001	0.000006	0.000000
0.34	2	0.001863	0.000003	0.000013	0.000001
0.34	4	0.003726	0.000006	0.000025	0.000002
0.34	6	0.005589	0.000009	0.000038	0.000002
0.34	12	0.011178	0.000018	0.000075	0.000005
0.34	24	0.022356	0.000035	0.000150	0.000010
0.34	52	0.048438	0.000076	0.000325	0.000021
0.34	104	0.096877	0.000152	0.000650	0.000042
0.34	156	0.145315	0.000228	0.000976	0.000062

Walleye (All Sizes) continued.

Upper Limit (95%) on Risk q* =.39 Mg/Kg/Day-1			Upper Limit (95%) on Risk q* =2.54 Mg/Kg/Day-1		
Mean	Max.	Min.	Mean	Max.	Min.
1.91E-07	8.18E-07	5.22E-08	1.25E-06	5.33E-06	3.40E-07
3.83E-07	1.64E-06	1.04E-07	2.49E-06	1.07E-05	6.80E-07
7.66E-07	3.27E-06	2.09E-07	4.99E-06	2.13E-05	1.36E-06
1.15E-06	4.91E-06	3.13E-07	7.48E-06	3.20E-05	2.04E-06
2.30E-06	9.81E-06	6.26E-07	1.50E-05	6.39E-05	4.08E-06
4.59E-06	1.96E-05	1.25E-06	2.99E-05	1.28E-04	8.16E-06
9.95E-06	4.25E-05	2.71E-06	6.48E-05	2.77E-04	1.77E-05
1.99E-05	8.51E-05	5.43E-06	1.30E-04	5.54E-04	3.54E-05
2.99E-05	1.28E-04	8.14E-06	1.94E-04	8.31E-04	5.30E-05
3.83E-07	1.64E-06	1.04E-07	2.49E-06	1.07E-05	6.80E-07
7.66E-07	3.27E-06	2.09E-07	4.99E-06	2.13E-05	1.36E-06
1.53E-06	6.54E-06	4.18E-07	9.97E-06	4.26E-05	2.72E-06
2.30E-06	9.81E-06	6.26E-07	1.50E-05	6.39E-05	4.08E-06
4.59E-06	1.96E-05	1.25E-06	2.99E-05	1.28E-04	8.16E-06
9.19E-06	3.93E-05	2.51E-06	5.98E-05	2.56E-04	1.63E-05
1.99E-05	8.51E-05	5.43E-06	1.30E-04	5.54E-04	3.54E-05
3.98E-05	1.70E-04	1.09E-05	2.59E-04	1.11E-03	7.07E-05
5.97E-05	2.55E-04	1.63E-05	3.89E-04	1.66E-03	1.06E-04
5.71E-07	2.44E-06	1.56E-07	3.72E-06	1.59E-05	1.01E-06
1.14E-06	4.88E-06	3.11E-07	7.44E-06	3.18E-05	2.03E-06
2.28E-06	9.76E-06	6.23E-07	1.49E-05	6.35E-05	4.06E-06
3.43E-06	1.46E-05	9.34E-07	2.23E-05	9.53E-05	6.08E-06
6.85E-06	2.93E-05	1.87E-06	4.46E-05	1.91E-04	1.22E-05
1.37E-05	5.85E-05	3.74E-06	8.92E-05	3.81E-04	2.43E-05
2.97E-05	1.27E-04	8.10E-06	1.93E-04	8.26E-04	5.27E-05
5.94E-05	2.54E-04	1.62E-05	3.87E-04	1.65E-03	1.05E-04
8.91E-05	3.81E-04	2.43E-05	5.80E-04	2.48E-03	1.58E-04

Walleye (All Sizes) continued.

Hazard Index		
RfD =	0.00046 Liver	
Mean Conc.	Max. Conc.	Min. Conc.
0.00	0.00	0.00
0.00	0.01	0.00
0.00	0.02	0.00
0.01	0.03	0.00
0.01	0.05	0.00
0.03	0.11	0.01
0.06	0.24	0.02
0.11	0.47	0.03
0.17	0.71	0.05
0.00	0.01	0.00
0.00	0.02	0.00
0.01	0.04	0.00
0.01	0.05	0.00
0.03	0.11	0.01
0.05	0.22	0.01
0.11	0.47	0.03
0.22	0.95	0.06
0.33	1.42	0.09
0.00	0.01	0.00
0.01	0.03	0.00
0.01	0.05	0.00
0.02	0.08	0.01
0.04	0.16	0.01
0.08	0.33	0.02
0.17	0.71	0.05
0.33	1.41	0.09
0.50	2.12	0.14

CHAPTER 6

HAZARD ASSESSMENT AND THE CHARACTERIZATION OF CARCINOGENIC AND OTHER RISKS TO HUMANS EXPOSED TO DIELDRIN

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INTRODUCTION

Dieldrin is a stable, persistent, bioaccumulative chemical that is widely distributed in the environment. Dieldrin and aldrin, which transforms to dieldrin in organisms or the environment, are cyclodiene insecticides used extensively in the United States from 1950 to 1974 when most uses were banned (U.S. EPA, 1987). Although no longer produced in the United States, existing stocks of dieldrin are registered for use in termite control by direct soil injection. Household use of existing supplies of aldrin and dieldrin is also legal (personal communication, U.S. EPA, 1989).

Chemical and Physical Properties

The water solubility of aldrin and dieldrin is 27 ug/l at 27^{o}C and 186 ug/l at 25^{o}C, respectively. The evaporation half-life (t1/2) of aldrin and dieldrin from water at a depth of one meter is 185 hours at 25^{o}C and 12,940 hours at 25^{o}C, respectively. A log octanol/water partition coefficient of 3.01 has been reported for aldrin (U.S. EPA, 1987). The value for dieldrin is 5.16 (Travis et al., 1988). The long half-life of dieldrin in water indicates a great potential for bioconcentration in aquatic organisms and subsequent exposures in wildlife and human populations consuming contaminated biota.

Human Exposure

Prior to the banning of most uses of aldrin and dieldrin, inhalation, skin absorption, and the ingestion of contaminated food and water were all important routes of exposure to these insecticides (U.S. EPA, 1987). Environmental concentrations have decreased since the early 1970's, and excluding exposures related to pesticide application, the consumption of contaminated food is the most important current route of exposure to dieldrin in the United States.

Air

Atmospheric concentrations were present in 85% of air samples taken by the U.S. EPA in 1976 up to 2.8 ng/m^3 resulting in an estimated intake of 0.098 ug/day. WHO (1984) reported a maximum air concentration in the United States of 29.7 ng/m^3. IARC estimated that the intake by occupationally exposed workers was from 0.72 to 1.1 mg/person/day compared to 0.025 mg/person/day for the general population during the late 1960's.

Water

In the 1960's, dieldrin was found in all major U.S. river basins more often than any other pesticide at a mean concentration of 7.5 ng/l (0.00075 ppb). In 1976, dieldrin concentrations ranging from 5 to 396 ng/l were reported for many surface waters, and from 1 to 7 ng/l in drinking water (U.S. EPA, 1987). The World Health Organization (WHO, 1984) concluded that pesticides in drinking water now account for 1% or less of the total exposure.

Food

According to the World Health Organization (WHO, 1984), ingestion of contaminated food is the most significant source of pesticide exposure. The overall dietary concentration of dieldrin was estimated to be 43 ng/g (ppb) in 1976 (U.S. EPA, 1987). Total dietary intake between 1965 and 1970 was reported to be 0.05 to 0.08 ug/kg body weight/day.

Data for adult market basket samples collected and analyzed between October 1980 and March 1982 were reported by Gartrell et al. (1986b). Dieldrin concentrations in all food groups ranged between trace to 11 ppb. Average daily intake of dieldrin from food was estimated to be 1.09 ug/day. Dieldrin was identified in dairy products, meat, fish and poultry, grain and cereal products, potatoes, leafy and root vegetables, garden fruits, and oils and fats. The highest percent of the average daily intake was accounted for by dairy products (0.454 ug/day, 41.7%), followed by meat, fish and poultry (0.304 ug/day, 28%). Garden fruits accounted for 14.5% (0.158 ug/day), and grain and cereal products (0.06 ug/day) and potatoes (0.06 ug/day) each accounted for 5.5% of the average daily intake in food. The total intake is equivalent to 0.016 ug/kg body weight/day. This intake level had remained essentially the same since 1978.

Pesticide residues in the food of infants (six month old) and toddlers (two years old) were reported for the same time period (Gartrell et al., 1986a). Dieldrin was identified in several components of the infant diet including whole milk, other dairy and dairy substitutes, meat, fish and poultry, potatoes, and vegetables. The average daily intake for all food was reported to be 0.163 ug/day. Sixty-six percent of the daily dieldrin intake was accounted for by whole milk (0.107 ug/day). Meat, fish and poultry accounted for 17% (0.027 ug/day), and other dairy products accounted for 12% (0.019 ug/day). Dieldrin was found in the toddler diet in whole milk, other dairy and dairy substitutes, meat, fish and poultry, potatoes, vegetables, and oils and fats. The average daily intake for all food was 0.309 ug/day. The food category, meat, fish and poultry, was the major contributor of dieldrin to the toddler diet. This category comprised 31% of total daily intake (0.0957 ug/day). Whole milk (0.757 ug/day) and other dairy products (0.0829 ug/day) accounted for 24.5% and 26.8%, respectively. Twelve percent of the daily intake was found in vegetables (0.0379 ug/day). The daily dieldrin intake was equivalent to an exposure of 0.02 ug/kg/day and 0.023 ug/kg/day in 8.2 kg infants and 13.7 kg toddlers, respectively. The daily dieldrin intake as a function of body weight in these age groups had decreased by approximately 50% since 1978.

Human Body Burden

Dieldrin concentrations in human adipose tissues sampled between 1976 and 1982 were 0.08 ppm (geometric mean) (Travis et al., 1988). Between 1963 to 1976 dieldrin residues in human adipose tissue had remained relatively constant at 0.15 to 0.22 ppm (Ackerman, 1980). The concentrations in the adipose tissue of three volunteers dosed 211 ug/day HEOD for 24 months ranged between 1.78 to 5 ppm. Subjects exposed to 0 or 10 ug/day HEOD for 18 months and then 211 ug/day for 6 months contained dieldrin concentrations in fat of 0.8 to 2.3 ppm. Exposure to 50 ug/day HEOD for 24 months resulted in adipose tissue concentrations of 0.6 to 1.6 ppm (Hunter et al., 1967; Hunter et al., 1969).

PHARMACODYNAMICS OF DIELDRIN

The pharmacodynamics of dieldrin have been studied in several mammalian species, including humans. Absorption, distribution, metabolism, and excretion of dieldrin is qualitatively similar, however, there are quantitative differences between strains and species. Sex differences are also apparent within particular animal strains.

HUMAN

Absorption and Distribution

Hunter et al. (1967, 1969) reported a study of human males given daily oral doses of dieldrin in gelatin capsules for 24 months. In a first experiment, the subjects, in groups of three, were given doses of 10, 50, and 211 ug HEOD per day for 18 months. A group of four control subjects were also included in the experiment. The concentration of HEOD in blood was measured monthly, and biopsies of subcutaneous fat were taken at month 3, 9, and 15.

A positive correlation was observed between HEOD concentration in blood and fat and the magnitude of the daily oral dose. In addition, a plot of the concentration of HEOD in blood versus the concentration in adipose tissue also appeared to be correlated. Individual regression lines of blood concentration versus adipose tissue concentration were significant for all individuals dosed with 211 ug/day and for two out of three of those dosed with 50 ug/day. The authors stated that this observation supports the concept of a dynamic equilibrium in the distribution of HEOD between blood and fat. The ratio of HEOD concentration in adipose tissue to concentration in blood was 156. After 15 months dieldrin levels in the blood of the group dosed 211 ug HEOD/day was 0.0143 ppm, and the concentration in adipose tissue was 2.12 ppm.

The relationship of blood HEOD concentration and adipose tissue concentration with time was also investigated. Blood concentrations increased by ten times in the 211 ug/day group and by four times in the 50 ug/day group after 15 months. Blood concentrations in the 10 ug/day group increased only slightly. The levels in blood were observed to level off with time, and the data for the 211 ug/day group were fitted to a quadratic equation. The quadratic coefficient was negative, and the fit of the polynomial was statistically significant. The authors proposed a model that would result in a balance between the amount of HEOD ingested and the amount eliminated. They predicted that this balance would occur after a period equal to four times the biological half-life of HEOD. A half-life for HEOD of between three and four months was stated to be consistent with the behavior of the compound in the blood of the subjects studied in this trial.

The attainment of an equilibrium between tissue concentration and HEOD elimination was investigated in individuals dosed 50 ug/day and 211 ug/day for another six months. The 10 ug/day group as well as the group of controls were also given 211 ug/day for an additional six months. Analysis of variance showed that the exponential model for dieldrin concentration in blood fit the data significantly better than the linear model in both dose groups. The exponential model for adipose tissue fit the data significantly better for the 211 ug/day group, but not the 50 ug/day group. The lack of significance in the lower dose group was attributed to the small number of observations. The authors concluded that the experimental evidence for humans indicated that the relationship between the concentration of HEOD in blood or adipose tissue and the time of exposure was curvilinear implying the approach of a steady state with time.

The correlation between blood and adipose tissue concentrations was confirmed and was characterized by the equation;

$$[A] = d[B]^m$$

where [A] is the concentration in adipose tissue and [B] is the concentration in blood. The parameter, m, was not significantly different from one. The value, d, was estimated to be 136 with confidence limits of 109 - 170.

Average daily intake for the general United Kingdom population and occupationally exposed populations was estimated using the quantitative relationship between blood or adipose tissue concentrations and the daily dose. The estimated occupational intake was 40 times higher than that of the general population.

Elimination

The rate of HEOD elimination was also studied by Hunter et al. Blood concentrations were monitored for eight months after dosing of the subjects was stopped, and the data were fitted to a simple exponential decline equation. A biological half-life of 369 days was calculated, 3 - 4 times the half-life estimated previously. The rate of decline of blood concentration was significantly different between individuals. Three of the subjects did not have a significant decline in blood HEOD levels. The biological half-life calculated from the other nine individuals ranged from 141 to 592 days.

The transfer of dieldrin from maternal tissues to infants is a route of elimination in humans. Dieldrin has been detected in breast milk, placental and meconium tissue, and the blood of infants.

The effect of maternal behaviors on blood concentrations was investigated. A study of organochlorine pesticide residues in the blood of women and their babies in the Netherlands found that slimming during pregnancy increased dieldrin concentrations in blood, but that breast feeding had no effect (Eckenhausen et al., 1981). Geometric mean concentrations for the entire sample were 0.8 ng/ml (ppb) (range 0.3-3.5) in prenatal maternal blood, 0.4 ng/ml (range <0.1-4.1) in post-natal maternal blood, 0.5 ng/ml (range < 0.1-2.6) in umbilical cord blood, and 0.3 ng/ml (range < 0.1-4.6) in babies' blood. The geometric mean concentrations of dieldrin in whole blood are shown in Table 1 for four categories according to whether or not the mothers dieted during pregnancy and whether or not the infants were breastfed.

There appears to be only a slight placental barrier to dieldrin. Maternal prenatal blood dieldrin levels were slightly higher than cord blood levels taken at parturition in all categories, except in slimming,non-breast feeding mothers. The geometric mean dieldrin concentration in placental tissue and meconium were 1.9 and 3.1 ng/ml, respectively. The presence of dieldrin in these samples indicates that the compound is transferred to the developing fetus via the placenta or by ingestion of amniotic fluid.

Nonslimming mothers had a decreased dieldrin concentration in the blood after birth. The reduction was significant only for breast-feeding mothers, however. At two weeks, babies with mothers in the slimming, breast feeding category had slightly higher blood dieldrin concentrations than the babies in the other categories. The difference in concentration was not statistically significant. Dieldrin concentrations in the blood of babies with nonslimming mothers were not different between breastfed and bottlefed infants. The babies of slimming, breast feeding mothers appeared to have higher blood concentrations than babies of slimming, non-breast feeding mothers, although the sample sizes precluded statistical evaluation. The geometric mean dieldrin concentration in breast milk was 2.3 ng/ml. In contrast to the authors conclusion, this data suggests that breast feeding is a possible factor in eliminating dieldrin from maternal tissues, however, the effect did not appear to be strong in this group.

The authors estimated that a baby's daily intake of dieldrin in breast milk during the first three months after birth could increase from 1.2 to 2.1 ug

(0.35 ug/kg body weight/day), given the mean dieldrin content in breast milk of 2.3 ng/ml. Elimination of dieldrin from a mother by lactation was estimated to increase from 12% of daily dieldrin intake at the birth of the child, to 21% at 3 months after birth.

Mothers that dieted during pregnancy had a higher post-natal blood concentration of dieldrin than mothers who did not (geometric mean: 1.0 versus 0.3 ng/ml, $p<0.05$). The babies' blood also corresponded to this behavior. Babies of slimming mothers had blood dieldrin levels of 0.7 ng/ml, while babies of nonslimming mothers had levels of 0.3 ng/ml. Losing weight appeared to promote the transfer of dieldrin into blood from adipose tissue and to increase the amount of dieldrin provided to the infant through the placenta.

The authors reported that they were not able to detect the metabolites of dieldrin, including the conjugated forms, in meconium or fecal samples in a preliminary study. The limit of detection was less than 1 ng/ml.

Table 1. Geometric Mean Concentrations of Dieldrin in Whole Blood by Maternal Behavior Category (Eckenhausen et al., 1981).

	Non-Slimming, Breast Feeding		Non-Slimming, Non-breast Feeding		Slimming, Breast Feeding		Slimming Non-breast Feeding	
Blood Sample	N	ng/ml	N	ng/ml	N	ng/ml	N	ng/ml
Maternal, prenatal	20	0.7	13	0.7	9	0.7	6	1.1
Maternal, postnatal								
2 wk	15	0.3	12	0.5	7	1.7	4	1.0
2 mo	11	0.4	8	0.4	3	0.8	1	-
3 mo	5	0.3	5	0.4	1	-	1	-
Cord Blood	19	0.5	13	0.5	7	0.5	4	1.2
Babies'								
2 wk	22	0.5	14	0.4	4	0.9	3	0.4
2 mo	17	0.4	10	0.3	2	1.4	-	-
3 mo	8	0.5	6	0.7	1	-	-	-
95% Confidence Limit(+ and -)*	20	1.4	13	1.6	7	1.7	4	2.6

*Confidence limits based on the specified sample size. The upper and lower limits are obtained by multiplying and dividing a mean concentration by the factor for each group.

PCB and organochlorine pesticide residue levels in the breast milk and maternal blood of 16 Canadian women during a three month lactation period were reported by Mes et al. (1984). The women resided in the National Capital Region, had a mean age of 35 years, ate less than one-half pound of mainly salt water fish per week and did not use pesticides. Milk samples were taken at eight points during the study, 7, 14, 28, 42, 56, 70, 84, and 98 days postpartum. Dieldrin concentrations in maternal blood were 0.1 to 0.2 ng/g (ppb) whole blood during the first 42 days after which only trace levels were detected. Dieldrin residues in breast milk ranged between 1.0 and 1.8 ng/g (ppb) whole milk during the 98 day lactation period, and concentrations were not significantly different at any sampling interval. No correlation was observed between milk and blood residue levels, either on whole or fat basis. The theoretical three month accumulation of dieldrin in infant adipose tissue was estimated and was the same order of magnitude as the dieldrin levels measured in the adipose tissues of Canadian adults. While pregnancy and breast feeding appeared to be a route of dieldrin elimination in women, in utero exposure and breast feeding were a significant source of dieldrin exposure to infants.

Dieldrin residues were detected in the urine of five men and five women of the general population with no occupational exposure to chlorinated insecticides (Cueto and Biros, 1967). Mean dieldrin concentrations were 0.0008 ± 0.0002 ppm in males and 0.0013 ± 0.0001 ppm in females. Urine specimens from men in a factory which made and formulated dieldrin contained dieldrin concentrations that increased with the level of exposure. There was also a significant difference between dieldrin levels in the urine of the occupationally exposed group and levels in the general population. Dieldrin concentrations in the low, medium, and high exposure categories were 0.0053, 0.0138, and 0.0514 ppm, respectively ($p<0.025$), seven, 17, and 64 times the urinary concentration in the general population.

EXPERIMENTAL ANIMALS

Absorption

Studies in rats indicate that dieldrin is absorbed slowly from the gastrointestinal tract after an oral dose and is transported to the liver via the portal venous system. Only six percent of a ^{36}C-dieldrin dose (25 mg/kg) injected orally into female albino rats remained in the stomach, small intestine and large intestine after 24 hours, while 30% of the injected dose was recovered in feces during the same period (Heath and Vandekar, 1964). High concentrations were present in the stomach, duodenal walls, and liver of male and female Sprague-Dawley rats one hour after a single dose of 150 ug ^{14}C-dieldrin (equivalent to 10 ppm in diet) was administered by stomach tube (Iatropoulos et al., 1975). After three hours, concentrations in the upper gastrointestinal tract had decreased by 80% (males) and 60% (females), and after 48 hours, levels had decreased by 98% in both sexes. A very small fraction of the absorbed dose (less than one-seventh) was found in lymph collected from cannulated rats over a 24 hour period indicating that the portal venous system is the major route of absorption from the gut (Heath and Vandekar, 1964). The absorption rate was influenced by the solvent used as a dosing vehicle (arachis oil or glycerol formal).

Distribution

Concentrations in lymph nodes and adipose tissue of both sexes of Sprague-Dawley rats given one dose of 150 ug ^{14}C-dieldrin by stomach tube began to increase after three hours and continued to rise over a 48 hour observation period (Iatropoulos et al., 1975). The lymphatic system may be a major transport pathway for the redistribution of unmetabolized dieldrin to fat. Concentrations in the brain, muscle, and fat of male Sprague-Dawley rats given one dose of 10 mg/kg dieldrin in corn oil by stomach tube were 0.3%, 3-4%, and 5% of the administered dose after the initial four hours (Hayes, 1974). The levels in brain gradually decreased, while the residue level in muscle remained constant for the remainder of the 48 hour observation period. On the other hand, concentrations in fat continued to increase during the first 24 hours to 50 - 60 % of the administered dose. Fat, therefore is an important sink for the redistribution of dieldrin and its metabolites from the brain.

After a long term exposure, concentrations are highest in adipose tissue. Dieldrin is also present in liver, brain and blood in decreasing concentrations in rats and dogs (Walker et al., 1969). A male rhesus monkey, dosed with dieldrin intravenously for 75 days, had highest concentrations in adipose tissue, bone marrow, liver, pancreas and adrenals in decreasing order of concentration (Muller et al., 1975). A female rhesus monkey, dosed orally, had the highest dieldrin concentrations in adipose tissue and bone marrow after 10 days. Levels were also high in liver, bile, thymus, lymph nodes, and adrenals. Wright et al. (1978) observed dose-dependent concentrations of dieldrin in the livers of male rhesus monkeys (0.22 - 23.25 ppm) fed diets containing various concentrations of dieldrin ranging from 0 to 5 ppm for 69 to 74 months. Dieldrin was located primarily in the liver microsomes.

A study that compared the total radioactivity in three tissues in the CFE rat, CF1 mouse, and LACG mouse, all males, given one dose of ^{14}C-dieldrin by stomach tube found the highest levels of radioactivity in adipose tissue (Hutson, 1976). More radioactivity was found in the liver than in the kidney in the two species of mice, whereas more radioactivity was found in the kidney, than in the liver in the CFE rats. The species difference appeared to be a result of the relative production of the metabolite, pentachloroketone, by rats and mice.

Dieldrin residues in the kidneys of female Sprague-Dawley rats given a single dose of 150 ug ^{14}C-dieldrin by stomach tube never exceeded 0.4 ppm and remained constant throughout a 48 hour period (Iatropoulos et al., 1975). In contrast, the concentration in the kidneys of males reached a peak at 5.1 ppm after five hours and then declined to 0.8 ppm at the end of 48 hours. Female rats may not have the capacity to metabolize dieldrin to pentachloroketone.

Sex differences among rats were also noted in the relative concentrations of residues in tissues. Female Carworth Farm E strain rats had tissue dieldrin levels two to ten times higher than those in male rats fed the same dietary concentration (Walker et al., 1969). The carcasses of female Sprague-Dawley rats fed 0.04, 0.2, or 2.0 ug/g ^{14}C-dieldrin in the diet for 273 days contained a higher proportion of the total dose ingested than the carcasses of males fed the same dietary levels (Davison, 1973). Another metabolic study using albino rats who received a single meal containing 10 ppm ^{14}C-dieldrin found that females had more radioactivity per gram in most organs and tissues than the male rats (Matthews et al., 1971). This pattern was not observed for the kidney and stomach. Residue levels in lungs and intestines were comparable betweem males and females.

Differences between sexes have also been reported for mice, but not for dogs. Dieldrin concentrations in the lipids of the total carcasses of Swiss-Webster mice fed diets containing 5 or 10 ppm aldrin over seven generations were higher in females than males (Deichmann et al., 1975). Tissue concentrations of beagle dogs fed dieldrin were similar between sexes, however. (Walker et al., 1969).

The metabolism studies generally measured concentrations of dieldrin residues in tissues that increased by an order of magnitude in blood, liver and fat, respectively. Lay et al. (1982) observed no significant difference between intraperitoneal (i.p.) and oral routes of administration in tissue distribution.

Tissue concentrations of dieldrin are associated with the level of daily intake. Walker et al. (1969) fed groups of Carworth Farm E strain rats (25 rats/sex/group) diets containing 0.1, 1.0, and 10.0 ppm dieldrin for two years. A control group of 45 rats per sex was also maintained for the two year period. Groups of five beagle dogs/sex/group were given dieldrin in capsules equivalent to 0.1 and 1.0 ppm or a control dose of olive oil for two years. A highly significant relationship between the level of intake and the concentration in whole-blood, brain, liver or adipose tissue was found in both rats and dogs. The relationship was linear when the data were transformed to logarithms, and the regression accounted for greater than 95% of the variation between doses. The relationship took the form;

$$c = aD^b$$

where c is tissue concentration, D is the amount ingested daily, and a and b are parameters describing the relationship.

The parameter, b, was found to be slightly less than one. The relationship was statistically significant in two cases involving rats, and in all but three of the cases involving dogs. This implies that the relationship between the untransformed variates is almost linear with a tendency to rise less steeply at higher intakes. The storage ratio, the ratio of tissue concentration to level of intake, was stated to be constant and equal to a when b is equal to unity. This is also approximately true if b is close to unity. The value of the parameter, a, for male and female rats was 1.91 and 8.8, respectively. The value of a for male and female dogs was 10 and 13.8, respectively.

The range of values for b calculated by Quaife et al. (1967) for the rat (0.62- 0.8) were lower than those estimated by Walker et al. (1969) (0.92 - 0.99). The estimates of b reported by Quaife et al. for four other species of cows, sheep, pigs, and hen ranged between 0.85 - 1.14 while Walker et al. calculated values between 0.79 and 0.99 for rats and dogs. Walker et al. concluded that the variation in the value of b between species were quite small given the heterogeneity of the experimental data.

An increase in dieldrin concentrations in tissues with increasing dose level was also observed in mice. Swiss-Webster mice were fed diets containing 5 or 10 ppm aldrin over seven generations (Deichmann et al., 1975). The parental generation was exposed beginning at weaning age while all other generations, except the F_4 generation, were exposed during gestation, via nursing, and in the diet. Dieldrin residues in abdominal fat and total extractable lipids were determined after 260 ± 15 days on the test diets. Total body lipids (g/mouse) were significantly increased ($p<0.05$) in the F1 - F3 generations compared to the parental generation, except in female control mice. Dieldrin concentrations also followed this pattern. Second generation mice (F1) fed aldrin 5 ppm and 10 ppm had mean dieldrin concentrations in the lipids of the entire carcass approximately twice the levels in the parental generation. Concentrations in parental and F1 generation males were 31 and 50 ppm, respectively, in the 5 ppm aldrin group, and were 60 and 91 ppm, respectively, in the 10 ppm aldrin group. Concentrations in parental and F1 generation females were 38 and 52 ppm, respectively, in the 5 ppm aldrin group, and were 60 and 145 ppm, respectively, in the 10 ppm aldrin group.

The concentration of dieldrin in tissues is related to the concentration in blood. Walker et al. (1969) found that the relationship was linear for adipose tissue and liver, but not the brain in rats and dogs. The authors concluded that a dynamic equilibrium existed between the blood and other tissues. The relationship takes the form;

$$y = dx^m$$

where y is the tissue concentration, and x is the blood concentration. The parameter, d, can be thought of as the ratio of tissue concentration to blood concentration. For adipose tissue this ratio was 104 and 137 for male and female rats, respectively, and 169 and 201 for male and female dogs.

Deichmann et al. (1968) calculated a fat to blood ratio of 474 for female Osborne-Mendel rats fed 50 ppm dieldrin for 183 days. The ratio represented the mean of all ratios calculated at various time points during the exposure period. After 183 days the fat to blood ratio was 684.

Tissue concentrations are related to the duration of exposure. HEOD levels in the blood and adipose tissue of rats fed 0, 0.1, and 1.0 ppm dieldrin over a two year period achieved a steady state at the end of six months (Walker et al., 1969). The upper limit was characteristic of the tissue and the amount of HEOD ingested per day. Dieldrin residues in the blood and livers of female Osborne-Mendel rats fed dietary concentrations of 50 ppm dieldrin for 183 days increased for nine days after which they remained constant for six months (Deichmann et al., 1968). Concentrations in adipose tissue were maintained with some fluctuation after the first 16 days.

Whole blood concentrations in beagle dogs given 0, 0.1, and 1.0 ppm dieldrin for two years also followed an asymptotic relationship over time during the first 18 months (Walker et al., 1968). Blood concentrations reached an equilibrium between weeks 18 and 30 in the high dose group and between weeks 12 and 18 in the low dose group. A significant deviation from this pattern was observed during the last six months, however, particularly in the high dose group.

The distribution of dieldrin between maternal and fetal tissues was studied in rabbits. Hathway et al. (1967) studied the distribution of [^{14}C]-dieldrin in the blood of pregnant New Zealand white rabbits and embryo/fetal tissues. Four periods during pregnancy were investigated, blastogenesis (6 days), implanted blastocyst invading the wall of the uterus (9 days), the small developing fetus with a fully established placenta (16 days), and the fetus in the last third of pregnancy with well-developed organs (24 days). Approximately 30 - 40 minutes after an intravenous injection of [^{14}C]-dieldrin into 6 day pregnant rabbits the concentration of [^{14}C]-dieldrin in free blastocysts began to equilibrate with the concentration in maternal blood. After 60 minutes, the concentration fell rapidly to a low level. The rate of uptake by 9 day implanted blastocysts was slow compared to the 6 day old blastocysts. Equilibration with maternal blood levels occurred after four hours.

[^{14}C]-dieldrin was not present in the allantoic and amnionic fluids of 16-day pregnant rabbits. Therefore, transfer of dieldrin to the fetus was concluded to be via the placenta. The maternal blood concentrations at different points during the course of two hours after an intravenous injection varied widely, and the spread of values in whole fetuses did not differ significantly from those in maternal blood. The time course for concentrations of [^{14}C]-dieldrin in maternal blood and 24-day fetal blood were parallel. The presence of

[^{14}C]-dieldrin in the blood of mothers and non-injected fetuses after the injection of a single fetus confirmed the suggestion that the transplacental transfer of dieldrin is two-way, at least during the last third of pregnancy.

Metabolism

Dieldrin is hydroxylated to 9-hydroxydieldrin by liver microsomal mono-oxygenase from rats (U.S. EPA, 1987). The addition of mono-oxygenase inhibitor sesamex inhibits the reaction (Matthews and Matsumara, 1969). This metabolite is conjugated with glucuronide acid *in vivo* and *in vitro*. The glucuronide was identified in the bile of rats (Heath and Vandekar, 1964; Chipman and Walker, 1979). Deconjugation occurs in the intestine and 9-hydroxydieldrin is excreted in the feces in the free form. Muller et al. (1975) reported the presence of 9-hydroxydieldrin in the urine of monkeys.

Dieldrin is also metabolized by epoxide hydratase to 6,7-trans-dihydroxydihydro-aldrin (U.S. EPA, 1987). This compound is further oxidized to aldrin dicarboxylic acid (ADA) or is conjugated by glucuronic acid. ADA was found in the urine of mice and rats (Baldwin et al., 1972; Hutson, 1976) and in the feces of rats (Hutson, 1976). The diol conjugate was identified in the urine of rabbits and monkeys (Muller et al., 1975, 1979).

Pentachloroketone (PCK), also known as Klein's metabolite, is a major urinary metabolite in male rats, but is found in trace amounts in the urine of female rats and male mice (Matthew et al, 1971; Baldwin et al., 1972; Hutson, 1976).

Metabolites in Tissues

Table 2 presents the percent composition of dieldrin and metabolites reported in adipose tissue, liver, and kidneys of rats and mice. Residues in adipose tissue are composed almost entirely of dieldrin in rats and mice, males and females. Dieldrin is also a major constituent in the liver, however species and sex differences are present. The male rat liver contained 21 - 31 percent pentachloroketone (PCK), whereas the PCK levels in the livers of CF-1 and LACG mice were very low or nondetectable. Moreover, the livers of female rats contained more than 99% unchanged dieldrin and no PCK. The metabolite, 9-hydroxydieldrin, comprised 37% of the dieldrin residues in the liver of male CF-1 mice, and 21% of the residues in the liver of male LACG mice. This metabolite was not present in male or female albino rats or CFE rats.

The species and sex differences noted in dieldrin residues in the liver were also present in the kidney of male and female rats and mice. Kidneys in both mice strains (CF-1 and LACG) and female albino rats contained 94 - 100% unchanged dieldrin. The kidneys of male CFE and albino rats contained 13 - 20% dieldrin and 78 - 86% PCK. These metabolites were not detected in the kidneys of female albino rats, male CF-1 mice, or male LACG mice.

The differences in tissue residues suggest diverging metabolic pathways between male and female rats, and between mice and male rats. Female albino rats appear to metabolize dieldrin in a manner similar to male mice.

Table 2. Percent Composition of Dieldrin and Metabolites in the Adipose Tissue, Liver, and Kidneys of Rats and Mice.

	Percent Composition		
	Adipose Tissue		
Species	Dieldrin	Polar Metabolite	PCK
Albino Rats[1]			
Male	> 99	< 1	3.5
Female	> 99	< 1	< 1

	Dieldrin	9-Hydroxy-dieldrin	PCK	Photo-dieldrin	Trans-dihydro-aldrindiol
CFE Rats[2]					
Male	98.4	< LOD	1.6	< LOD	< LOD
CF1 Mice					
Male	100.0	< LOD	< LOD	< LOD	< LOD
LACG Mice					
Male	99.0	0.5	< LOD	< LOD	< LOD

	Liver		
Species	Dieldrin	Polar Metabolite	PCK
Albino Rats[1]			
Male	68.0	< 1	31.2
Female	> 99	< 1	< 1

	Dieldrin	9-Hydroxy-dieldrin	PCK	Photo-dieldrin	Trans-dihydro-aldrindiol
CFE Rats[2]					
Male	79.0	< LOD	21.0	< LOD	< LOD
CF-1 Mice					
Male	60.0	37.0	3.6	< LOD	< LOD
LACG Mice					
Male	79.0	21.0	< LOD	< LOD	< LOD

Table 2 continued.

	Kidney		
Species	Dieldrin	Polar Metabolite	Klein's Metabolite
Albino Rats[1]			
Male	13.0	< 1	85.6
Female	> 99	< 1	< 1

	Dieldrin	9-Hydroxy-dieldrin	PCK	Photo-dieldrin	Trans-dihydro-aldrindiol
CFE Rats[2]					
Male	20.4	1.6	78.0	< LOD	< LOD
CF-1 Mice					
Male	100.0	< LOD	< LOD	< LOD	< LOD
LACG Mice					
Male	94.0	3.0	< LOD	< LOD	< LOD

[1] Matthew et al., 1974

[2] Hutson, 1976

Elimination

Robinson et al. (1969) measured the decline in tissue concentrations of male CFE rats exposed to 10 ppm HEOD in the diet for eight weeks and then fed an untreated diet for up to 12 weeks. The data for liver and blood were fitted to regression equations with two exponential terms suggesting a rapid and slower elimination phase. The half-life of elimination for the rapid phase was 1.2 days and was 10.2 days for the slower phase. The decay constants calculated for blood were similar to those estimated for liver elimination. The change in concentration in adipose tissue was adequately accounted for by a single exponential type relationship. The half-life for elimination in adipose tissue was 10.3 days. A compartmental model was offered as a theoretical construct for the authors' empirical observations with a central compartment including blood and liver and a peripheral compartment including adipose tissue. The similarity of the exponential terms for the slower elimination phase in blood (-0.053 ± 0.003) and liver (-0.068 ± 0.006) and the term for adipose tissue (-.067 ± 0.002) led the authors to suggest that the rate limiting factor for whole body elimination is the transfer from the peripheral compartment to the central compartment.

An initial fast elimination phase, followed after six or seven days by a second slower elimination phase, was reported for the excretion of dieldrin residues by male albino rats (six rats per group) dosed daily for five days with 1.0, 0.1, or 0.01 mg/kg body weight/day (Lay et al., 1982). Groups of rats were dosed either orally or intraperitoneally. The mean total urinary and fecal excretion for all six groups combined was 78.8 ± 3.3% of the total administered dose 14 days after the last dose was administered. There were no significant differences in the extent of excretion between the two routes of administration. The radioactivity in urine was ten times less than that in feces. The amount of excretion was largely proportional to the administered dose. No acceleration of elimination was observed in the higher dose groups. This was interpreted to mean that liver enzyme induction had not occurred.

A female rhesus monkey also exhibited a rapid and slow elimination of dieldrin residues after a single intravenous injection of 2.5 mg/kg body weight (Muller et al., 1975a). During the first five days after dosing, 13% of the administered dose was excreted. A total of 20% of the administered dose was excreted by day 75. During the initial five days after dosing, 90% of the excreted radioactivity was found in feces and 10% was found in urine. By day 20, the percent of the daily excreted radioactivity found in urine had increased to 30 to 40%. Two male rhesus monkeys were given one oral dose of

0.5 or 0.36 mg/kg body weight and monitored for 10 days. At the end of the 10 days, 25.3% and 16.7% of the administered dose had been excreted by the monkeys dosed with 0.5 mg/kg and 0.36 mg/kg, respectively. The faster rate of elimination reported for the male monkeys dosed orally may be attributed either to sex differences or the different routes of administration.

Various studies have estimated a half-life of excretion of 7 to 11 days for male rats (Ludwig et al., 1964; Matthews et al., 1971; Chipman and Walker, 1979). The excretion half-life in female albino rats is greater than 21 to 30 days (Matthews et al., 1971).

Differences were observed between male and female albino rats in the excretion of dieldrin and its metabolites in feces and urine (Matthews et al., 1971). Males and females excreted approximately ten times more radioactivity in feces than in urine. Male feces contained three to four times more radioactivity than female feces. The parent compound represented only a low percent of the total radioactivity in the feces of male rats, whereas unchanged dieldrin initially comprised greater than 60% of the radioactivity in the feces of females. The parent compound continued to represent approximately 20% of the radioactivity in the feces of females throughout the experiment. Nine days after dosing, the excretion of dieldrin in the feces of males and females comprised 0.65% and 2.45%, respectively, of the total administered radioactivity. Two polar metabolites were present in significant amounts in the feces of males during the first four days after dosing (8 - 18%). The proportion of radioactivity in feces comprised by the polar metabolites decreased to only a small percentage of the total in males after the fourth day (7.8%) and in females at all time points (1 - 5%). The metabolite, 9-hydroxydieldrin was the major compound in feces in both males and females, representing 21.5% and 5.8% of the administered dose after nine days.

Radioactivity was 3 - 4 times higher in the urine of male rats than females. PCK represented more than 70% of the radioactivity on the first day after dosing, and comprised 97% or more by the sixth day. The remainder was identified as dieldrin with traces of trans-dihydroxy-dihydroaldrin. In females, dieldrin was present (20% of the radioactivity) during the first two days, with little or no dieldrin detected thereafter. The majority of the radioactivity was trans-dihydroxy-dihydroaldrin with only a trace of PCK.

Tables 3 and 4 compare the extent of excretion of radiolabelled dieldrin and metabolites at various time points after a single dose in different species and strains. Approximately 30 - 50% of the administered dose was measured in the feces of three strains of mice 8 - 10 days after dosing. Rats exhibited differences among strains and between sexes. Male CFE rats excreted 43 - 62% of the dose by 8 days, whereas male and female Sprague-Dawley rats excreted 9 - 11% of the dose after 10 days. Female albino rats also excreted a relatively small percent of the dose after 10 days (8.3%). On the other hand, male albino rats excreted approximately 25% of the dose in 10 days, an amount approaching that of male LACG mice. New Zealand white rabbits excreted a low proportion of the dose in feces by 10 days after dosing. The male rhesus monkey excreted 16% of the dose by 10 days, a lesser amount than the mice and rat strains except for the Sprague-Dawley rat strain. The female chimpanzee excreted only 5% of the dose in 10 days, a lower proportion than even the Sprague-Dawley strain. Sex differences in the rate of the excretion of radioactivity were noted in the albino rats and perhaps in the primates, but not in Sprague-Dawley rats, Swiss White mice, or New Zealand White rabbits.

Excretion in urine is approximately ten times lower than excretion in feces by rats and mice. The excretion of dieldrin residues in the urine of CFE male rats appears comparable although slightly higher than CF-1 male mice. The proportion of the dose excreted in the urine of Sprague-Dawley rats and male LACG mice is much lower.

Excretion in the urine of nonhuman primates is 4 - 5 times lower than excretion in feces. The proportion of the administered radioactivity found in the urine of the male rhesus monkey ten days after dosing was comparable to that found in the urine of male CFE rats eight days after a single dose, whereas the proportion in the urine of the female chimpanzee was lower.

In contrast to the pattern of excretion observed in rats, mice, and nonhuman primates, urine is a more important route of excretion in rabbits. Excretion in the urine of New Zealand White rabbits was 4 - 5 times higher than excretion in feces (Muller et al., 1975b). Rabbits dosed daily with 56 - 58 mg/kg body weight dieldrin-^{14}C by gastric intubation for 22 weeks excreted a proportion of the administered dose in urine that was twice the proportion excreted in feces (29.7% versus 12.7%). At 52 weeks, 20 weeks after dosing was discontinued, excretion in urine was three times higher than excretion in feces (43.1% of the administered radioactivity versus 12.6%). The overall excretion of dieldrin by rabbits is slower than rats and mice.

Table 3. Excretion of Radiolabelled Dieldrin and Metabolites In Feces After a Single Dose - Comparison of Species and Strains.

	Percent of Administered Dose					
	Days					
Species	1	7	8	10	14	28
Rats						
CFE male[1]			62.4			
CFE male[2]			42.9			
Sprague-Dawley						
male[3]				11.3		
female[3]				9.3		
Albino						
male[4]	3.0	21.4		24.5		
female[4]	0.6	7.3		8.3		
female[5]	30.0					70.0
Mice						
CF-1 male[1]		51.4				
CF-1 male[2]	51.7/ 64.5					
LACG male[1]		27.4				
Swiss White						
male[3]			36.6			
female[3]			35.0			
Rabbit						
New Zealand White						
male[3]			0.3			
female[3]			0.5			
Primates						
Rhesus						
male[3]			16.0			
Chimpanzee						
female[3]			5.0			

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[1] Hutson, 1976
[2] Baldwin and Robinson, 1972
[3] Muller et al., 1975b
[4] Matthews et al., 1971
[5] Heath and Vandekar, 1964

Table 4. Excretion of Radiolabelled Dieldrin and Metabolites In Urine After a Single Dose - Comparison of Species and Strains.

	Percent of Administered Dose				
	Days				
Species	7	8	10	14	28
Rats					
CFE male[1]		6.6			
CFE male[2]		4.2			
Sprague-Dawley					
male[3]			0.6		
female[3]			0.5		
Albino					
female[4]					10.0
Mice					
CF-1 male[1]		2.6			
CF-1 male[2]	4.6/ 5.9				
LACG male[1]		0.7			
Swiss White					
male[3]			1.9		
female[3]			1.9		
Rabbit					
New Zealand White					
male[3]			1.5		
female[3]			2.2		
Primates					
Rhesus					
male[3]			4.4		
Chimpanzee					
female[3]			1.3		

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[1] Hutson, 1976
[2] Baldwin and Robinson, 1972
[3] Muller et al., 1975b
[4] Heath and Vandekar, 1964

The radioactivity in the excreta of male albino rats (six rats per group) dosed daily either orally or intraperitoneally for five days with 1.0, 0.1, or 0.01 mg/kg body weight/day was composed of dieldrin, syn-9-hydroxy-dieldrin, trans-4,5-dihydroxy-dihydroaldrin, and conjugates of the diol (Lay et al., 1982). The relative proportions of metabolites were not changed by different doses or routes of administration. The percent composition of metabolites in feces in the rats dosed with 10 ug/kg body weight were 13.5% conjugates, 64.2% 9-hydroxydieldrin, 6.8% trans-4,5-dihydroxy-dihydroaldrin, and 15.5% dieldrin. The percent composition in urine in the same dose group was 27.1% conjugates, 56.7% 9-hydroxydieldrin, 9.3% trans-4,5-dihydroxy-dihydroaldrin, and 6.9% dieldrin.

The same metabolites were identified in the excreta of rhesus monkeys dosed with one i.v. injection or orally (Muller et al., 1975a). The metabolites, 9-hydroxydieldrin, 4,5-aldrin-trans-dihydro-diol, and the glucuronic acid conjugate of the diol, were the major components in excreta in the female dosed by i.v. injection. During the first five days after dosing, the conjugate and 9-hydroxydieldrin each comprised 40% of the excreted radioactivity. By day 20, 9-hydroxydieldrin represented greater than 95% of the daily excretion. Only unchanged dieldrin was identified in the excreta of male rhesus monkeys dosed orally during the first two days after treatment. However in urine, and in feces after the third day, the same metabolites were identified as in the excreta of the female monkey dosed intravenously. After 10 days, unchanged dieldrin and 9-hydroxydieldrin each represented greater than 40% of the excreted radioactivity. The major metabolism pathway for dieldrin appears to be different in rabbits. Trans-6,7-dihydro-xy-dihydroaldrin comprised 86% of the total activity found in the urine of rabbits dosed for 21 weeks with 56 - 58 mg/kg dieldrin-^{14}C (Korte and Arent, 1968).

Tables 5, 6, and 7 present comparative data generated by Hutson, 1976 and Muller et al. (1975b) of the excretion of metabolites in feces and urine by various animal species. Tables 5 and 6 show that 9-hydroxydieldrin is the major metabolite in the excreta of the CFE rat and Sprague-Dawley rat. The percent of the administered dose represented by 9-hydroxydieldrin and unchanged dieldrin was about equal in the excreta of male CF-1 mice, male LACG mice, male rhesus monkey, and female chimpanzee. The proportion of the dose comprised by 9-hydroxydieldrin in feces increased from 13% to 20% in male CF-1 mice and from 5% to more than 20% in male LACG mice that were pretreated with dieldrin for four weeks prior to a single dose of radiolabelled dieldrin. On the other hand, the trans-dihydro-aldrindiol was the major metabolite in the excreta of Swiss White mice and New Zealand White rabbits.

Dieldrin and pentachloroketone comprised the majority of the radioactivity in the urine of male CFE rats one to two days after a single dose of dieldrin-^{14}C. Polar metabolites were present in minimal amounts (Table 7). The urine of CF-1 mice and LACG mice had a greater percentage of polar metabolites and a lesser percentage of dieldrin and pentachloroketone. The reversed composition was more marked in the LACG mice.

Table 5. Metabolites in Excreta of Different Species Ten Days After a Single Oral Dose of 0.5 mg/kg Dieldrin-^{14}C (Muller et al., 1975b).

	Percent of Administered Dose							
	Swiss White Mice		Sprague-Dawley Rats		New Zealand White Rabbits		Rhesus Monkeys	Chimpanzees
	M	F	M	F	M	F	M	F
Dieldrin	5.5	3.2	0.8	2.8	0.3	0.5	9.0	3.2
9-Hydroxy-Dieldrin	13.0	7.5	8.8	4.6	--	0.2	9.4	2.0
Tr-Aldrin-Diol	20.0	26.0	2.3	2.4	1.5	2.0	2.0	1.1

Table 6. Metabolites in Feces (Percent of Administered Dose) Seven Days After a Single Dose of Dieldrin-^{14}C (Hutson, 1976).

	Percent of Administered Dose					
	CFE Rat, Male		CF-1 Mouse, Male		LACG Mouse, Male	
	Normal	Pretreated	Normal	Pretreated	Normal	Pretreated
Dieldrin	6-7%	8-9%	> 15%	10%	8%	8%
9-Hydroxy-Dieldrin	> 30%	> 30%	13%	20%	5%	> 20%
4,5-Trans-Dihydro-Aldrindiol	< 5%	5%	7%	7%	< 5%	> 5%

Table 7. Metabolites in Urine (Percent Composition) One or Two Days After a Single Dose of Dieldrin-^{14}C (Hutson, 1976).

	CFE Rats	CF-1 Mice	LACG Mice
Dieldrin + PCK	80%	> 40-48%	< 17%
ADA	15%	3-4%	8-25%
Polar Metabolites	1%	48-56%	58-88%

Approximately ten times more radiolabelled dieldrin residues were excreted in feces than urine in albino rats (three males and females per group) receiving a single meal containing 10 ppm dieldrin-^{14}C (Matthews et al., 1971). Radioactivity in male feces and urine was three to four times higher than in female feces.

Data on dieldrin excretion in animals given repeated doses of the chemical are limited. Excretion was measured in Sprague-Dawley rats (two rats/sex/group) fed 0.04, 0.2 or 2.0 ug/g dieldrin-^{14}C in the diet for 273 days (Davison, 1973). The excretion of ^{14}C in feces and urine was significantly higher ($p<0.01$) in males than females through the fifth or sixth week. Maximal excretion of ^{14}C occurred by the sixth week in both sexes when excretion approximated the daily consumption of dieldrin. Females excreted a smaller proportion of the dose of ^{14}C in feces than male rats while the ^{14}C excreted in urine was similar in males and females. At the end of the dosing period males excreted 76.9 - 82.7% of the daily dose in feces and 10.4 - 13.3% of the dose in urine. Females excreted 71.6 - 76.1% of the dose in feces and 11.9 - 14.3% of the dose in urine.

Whereas the excretion of dieldrin residues did not quite balance the daily dose by 39 weeks in Sprague-Dawley rats, excretion by male Wistar strain rats (5 animals) given a daily dose of 4.3 ug aldrin-^{14}C by stomach tube (approximately 0.2 ppm in diet) was equal to the daily dose after 8 weeks of the 12 week dosing period (Ludwig et al., 1964). Eighty-two days after the last dose was administered, 99.54% of the total radioactivity administered had been excreted in feces and urine.

Daily excretion never equalled the daily dose administered in rhesus monkeys fed diets containing 2 ppm dieldrin for 260 days. The authors estimated that a steady state would have been reached after 350 to 400 days of dosing (Muller et al., 1979). After 260 days, the concentration in the fat of one male and one female monkey was 54.4 and 6.1 ppm dieldrin, respectively. Subsequently, after 130 days on a normal diet dieldrin levels were 21.4 ppm in males and 13 ppm in females.

CONCLUSION

The pharmacodynamics of dieldrin have been studied in several mammalian species, including humans. Absorption, distribution, metabolism, and excretion of dieldrin are qualitatively similar, but there are quantitative differences between strains and species. Sex differences are also apparent within particular animal strains.

The accumulation of dieldrin residues in blood and adipose tissue is dose-related in humans (Hunter et al., 1967, 1969) and in rats and dogs (Walker et al., 1969). The tissue concentration data in humans and animal species exposed to dieldrin for several months followed an exponential relationship to time suggesting the eventual attainment of a steady state. Hunter et al. predicted that an equilibrium between accumulation and elimination would occur 3 - 4 months after the estimated half-life. Walker et al. reported the achievement of a steady state in HEOD concentrations in blood and fat of CFE strain rats after six months and in the blood of beagle dogs after four to eight months. The time to the achievement of equilibrium was also dependent on the level of dieldrin intake. The excretion of dieldrin residues did not balance the daily dose after 39 weeks of dosing in Sprague-Dawley rats fed 0.04, 0.2, or 2.0 mg/kg (Davison, 1973). On the other hand, male Wistar strain rats dosed daily with 4.3 ug aldrin-^{14}C by stomach tube had reached a steady state by eight weeks (Ludwig et al., 1964). Rhesus monkeys did not reach a steady state during 260 days exposure 2.0 ppm dieldrin in the diet (Muller et al., 1979).

Adipose tissue is the major storage site in mammalian tissues, and a dynamic equilibrium between blood and adipose tissue levels of dieldrin is maintained. The ratio of HEOD concentration in adipose tissue to the concentration in blood has been calculated to be 156 in human males (Hunter et al., 1969), 104 and 137 in male and female rats (Walker et al., 1969), and 169 and 201 in male and female dogs (Walker et al., 1969).

Significant concentrations are also present in the liver in rats and dogs (Walker et al., 1969) and mice (Hutson, 1976). Dieldrin residues were also found in the kidneys of male CFE rats (Hutson, 1976). Adipose tissue, bone marrow, liver, pancreas, adrenals, thymus and lymph nodes were sites of dieldrin accumulation in decreasing order of importance in the rhesus monkey (Muller et al., 1975).

The pattern of distribution noted in the tissues of rats, mice, and monkeys, and the similar fat to blood ratios reported in humans, rats, and dogs, suggests that the distribution of dieldrin in the bodies of humans is similar. In general, dieldrin concentrations increased by an order of magnitude between blood and liver, and between liver and fat.

Sex differences among rats were noted in the relative concentrations of residues in tissues. Female Carworth Farm E strain rats had tissue dieldrin levels two to ten times higher than those in male rats fed the same dietary concentration (Walker et al., 1969). The carcasses of female Sprague-Dawley rats fed 0.04, 0.2, or 2.0 ug/g ^{14}C-dieldrin in the diet for 273 days contained a higher proportion of the total dose ingested than the carcasses of males fed the same dietary levels (Davison, 1973). Another metabolic study using albino rats who received a single meal containing 10 ppm ^{14}C-dieldrin found that females had more radioactivity per gram in most organs and tissues than the male rats (Matthews et al., 1971). Dieldrin concentrations in the lipids of the total carcasses of Swiss-Webster mice fed diets containing 5 or 10 ppm aldrin over seven generations were higher in females than males (Deichmann et al., 1975). Tissue concentrations of beagle dogs fed dieldrin were similar between sexes, however. (Walker et al., 1969).

The same metabolites are produced by all species, however, there are differences between species and between sexes in the relative importance of certain metabolites (Matthew et al., 1974; Hutson, 1976). Dieldrin was the predominant residue found in the adipose tissue of various strains of rats and mice. Unchanged dieldrin comprised a major proportion of the residues present in liver (60 - 99%). PCK is present in significant amounts in the livers of male rats (21 - 31%), whereas greater than 99% of the residue in the livers of female rats was unchanged dieldrin. On the other hand, 21 - 37% of the residues in mice livers was 9-hydroxydieldrin. Unchanged dieldrin was the predominant residue in the kidneys of male mice, while in rats, 80% of the residue was comprised of PCK. Clearly there are differences in the relative importance of metabolic pathways between rats and mice and between male rats and female rats.

The pattern of elimination of dieldrin is similar between species. Excretion in feces is the predominant route of elimination in all species, except rabbits. Excretion in feces is approximately ten times higher than in urine in rats and mice. Excretion in the feces of monkeys is 4 - 5 times the excretion in urine. In general, data in rats (Robinson et al., 1969; Lay et al., 1982) and monkeys (Muller et al., 1975a) suggest a slower elimination phase and a rapid elimination phase for the excretion of dieldrin residues in feces.

Various studies have estimated a half-life of excretion of 7 to 11 days for male rats (Ludwig et al., 1964; Matthews et al., 1971; Chipman and Walker, 1979). The excretion half-life in female albino rats is greater than 21 to 30 days (Matthews et al., 1971). The excretion half-life for humans appears to be longer than for the experimental animals studied. Chipman and Walker (1979) reported a half-life of excretion for humans of 270 days. Hunter et al. (1969) calculated a biological half-life for dieldrin in humans of 369 days based on the rate of decline of concentrations in blood. The range among subjects was 141 to 592 days, although three individuals among the 12 study participants showed no significant decline in blood HEOD levels. Moriarty (1975) estimated a half-life of about 2150 days for dieldrin in the plasma of humans.

A comparison of Tables 5 and 6 describing the percent of the administered dose excreted in feces at various times after a single dose of dieldrin indicates that excretion is fastest in male CFE rats and male CF-1 mice. The rate of excretion in male albino rats, male LACG mice, and male and female Swiss White mice is comparable, while that in male and female Sprague-Dawley rats is much slower. Fecal excretion in monkeys was also slower than in rats and mice.

Dieldrin and 9-hydroxydieldrin were the major components of residues present in the feces of rats, mice, and monkeys. Dieldrin and PCK comprised the majority of the residues present in the urine of male rats after a single dose, while mice had a greater proportion of polar metabolites. Other than the differences in the pattern of metabolites observed in the urine of rats and mice, there appears to be no pattern of metabolism that is unique to a particular species. Urinary excretion is a minor route for CFE rats, CF-1 mice, and LACG mice. The same metabolites were found in the urine of all species and strains, although in varied amounts.

SYSTEMIC TOXICITY OF DIELDRIN

Central nervous system intoxication occurs in all species, including humans, exposed to relatively high levels of dieldrin. Rodents are susceptible to liver toxicity at low doses of the chemical. Evidence of hepatic enzyme induction has been reported in monkeys and dogs chronically exposed to low dieldrin concentrations. Two investigations of toxicity in humans found no evidence of hepatic involvement in workers exposed from four to 15 years, or in volunteers exposed for two years. Other effects on the kidney, endocrine system, immune system, and central nervous system have been reported in moderately exposed experimental animals. The effect of long term exposure to low dieldrin concentrations on these other systems has not been fully evaluated.

TOXICITY IN EXPOSED HUMANS

The toxic effects of pesticide exposure in industrial workers in a manufacturing and formulating plant in Pernis, Holland were reported by Van Raalte (1977) and Jager (1970). In a total population of dieldrin-exposed workers, 32 dieldrin-involved cases of clinical intoxication, 19 of which included convulsions, were reported during 1954 to 1967. The average dieldrin concentration in blood at the time of convulsion was 280 to 290 ppb. A no effect level was established at 200 ppb in blood equivalent to an oral intake of 33 ug/kg/day.

Long term toxic effects were studied in 1968 among 233 of the dieldrin exposed men with four to 15 years of exposure. The values of various physical and hematologic parameters in the exposed workers were not found to be different from control values. In addition, three tests for enzyme induction - concentrations of DDE in the blood, the ratio of 6-B-hydroxycortisol to 17-OH-corticosteroids in the urine, and excretion of D-glucaric acid in the urine - found no evidence of elevated enzyme activity in workers with the highest dieldrin concentrations in blood compared to controls. Plasma alkaline phosphatase activity was not elevated in individuals intentionally dosed with up to 211 ug HEOD per day or 3 ug/kg/day for two years (Hunter and Robinson, 1967; Hunter et al., 1969).

Twenty-one workers or family members who were exposed to dieldrin contaminated poultry and eggs were studied (Bell and MacLeod, 1983). All had elevated blood dieldrin levels ranging from 0.002 to 0.016 ug/ml compared to 0.0005 ug/ml in the blood of patients in an area hospital. Six of the workers were found to have raised alkaline phosphatase levels. Blood dieldrin concentrations in this group ranged from 0.003 to 0.011 ug/ml (3 to 11 ppb). A maximum daily intake of dieldrin was estimated to be 3.8 mg. Dieldrin levels in blood taken one year after the original analysis from 12 of the 15 workers had fallen to approximately one-half of the original level.

SYSTEMIC TOXICITY IN EXPERIMENTAL ANIMALS

Table 8, from the U.S. EPA Carcinogenicity Assessment of Aldrin and Dieldrin (1987) presents the results of the long-term studies of dieldrin exposure in mice, rats, and dogs.

Mortality and Morbidity

Osborne-Mendel rats, 12/sex/dose group, were fed 0, 0.5, 2, 10, 50, 100, and 150 ppm dieldrin in the diet for two years (Fitzhugh et al., 1964). A dose-related decrease in survival was observed that became significant at 50 ppm and higher at 24 months (p=0.01). Mortality, body weight, food intake and general health were not affected in Carworth Farm E strain rats, 25/sex/dose group, fed 0.1, 1.0, and 10.0 ppm HEOD for two years. Irritability, tremors, and occasional convulsions were reported in the 10 ppm dose groups but not at lower doses.

The lifespan of Osborne-Mendel female rats was affected at dietary dose levels of 30 and 50 ppm technical dieldrin (Deichmann et al., 1970). Although body weights were not changed, mean survival of the females in these dose groups was decreased by two months. Survival and body weight in males was not affected. A dose-related incidence of tremors and clonic convulsions was also noted, primarily in females.

The lifespans of male and female GF-1 mice were shortened by the consumption 20 and 10 ppm dieldrin in their diets (Walker et al., 1972). During the first three months of treatment, 25% of males and 50% of females fed 20 ppm dieldrin died primarily of dieldrin intoxication. Liver enlargements were detected in male and female mice fed 20 ppm dieldrin in their diets after 36 weeks of treatment. All animals were autopsied by 12 months. This is not a true indication of survival because the study design involved the autopsy of healthy animals with intra-abdominal masses. Fifty-percent of the mice fed 10 ppm dieldrin had died or were killed by the fifteenth month in one experiment, and most were autopsied by 16 to 17 months in a second experiment. Intra-abdominal masses were palpated in males and females after 40 weeks. Intra-abdominal masses were detected in the mice fed 5 ppm and 2.5 ppm after 75 weeks and 100 weeks, respectively. Morbidity was reported to be slightly increased in these two groups. Morbidity was similar to controls in mice fed 0.1, 1.0 or 1.25 ppm dieldrin and intra-abdominal masses were not palpated.

Table 8. Summary of Long Term Studies of Dieldrin Toxicity in Experimental Animals from U.S. EPA (1987).

TABLE 4-4. (continued)

Species	Strain	No./Sex	Dose	Exposure	Effects	Reference
Rat	CF	25/M,F	0.1, 1, 10 ppm	2 yr	Irritability, tremors, and convulsions; increase in liver to body wt. ratio in the 1 and 10 ppm females; CHIRL observed at the 10 ppm; chronic nephritis and myocardial fibrosis in all groups.	Walker et al. (1969)
Rat	Osborne-Mendel	50/M,F	20/30, 50 ppm	29 mo	Dose-related tremors and clonic convulsions especially in females; lower survival for females at 30 and 50 ppm; significant increase in liver/body wt. ratio at 30 ppm; moderate (not dose related) increase in the incidence of hepatic centrilobular cloudy swelling and necrosis in both sexes at all levels.	Deichmann et al. (1970)
Rat	Fischer-344	24/M,F	2, 10, 50 ppm	2 yr	Central nervous system disorders (convulsions, tremors, nervous behavior) in high-dose males and females at 80 weeks.	NCI (1978b)
Rat	Osborne-Mendel	50/M,F	29 ppm 65 ppm	80 wk 59 wk	Organochlorine intoxication (tumors, convulsions, hyperexcitability) during the second year.	NCI (1978b)
Rat	Osborne-Mendel	12/M,F	0.5, 1, 2, 10, 50, 100, 150 ppm	2 yr	Decreased survival in rats fed at 50 ppm and above; 42% at 150 ppm dead by the end of one year; female rats died of renal hepatic necrosis; males had higher incidence of chronic nephritis at the 100 and 150 ppm levels.	Reuber (1980)

(continued on the following page)

Table 8 continued.

TABLE 4-4. TOXICITY OF DIELDRIN FOLLOWING LONG-TERM FEEDING TO MICE, RATS, AND DOGS

Species	Strain	No./Sex	Dose	Exposure	Effects	Reference
Mouse	C3HeB/Fe	100/M,F	10 ppm	2 yr	Reduced lifespan	Davis and Fitzhugh (1962)
Mouse	C3HeB/Fe	100/M,F	10 ppm	2 yr	None	Davis (1965)
Mouse	CF-1	125/M,F	0.1, 1	2 yr	None	Walker et al. (1972)
		200/M,F	10 ppm	2 yr	Decreased survival rate; palpable intra-abdominal masses after 9 months; half the males and females died or were sacrificed by the 15th month.	
Mouse	CF-1	30/M,F	10 ppm	2 yr	Increased mortality and enlarged liver in both sexes	Thorpe and Walker (1973)
Mouse	CD-1	100/M,F	0.15-15 ppm	25 mo	Time- and dose-related hepatic nodules	Benitz et al. (1977)
Mouse	C3HeB/Fe	100/M,F	10 ppm	2 yr	Hepatic vein thrombosis	Reuber (1977)
Mouse	B6C3F1	50/M,F	2.5, 5 ppm	80 wk	Tremors, irritability, abdominal distension	NCI (1978b)
Rat	CF-1	20/M,F	2.5, 12.5, 25 ppm	2 yr	Increase in liver/body wt. ratio in both sexes at 2.5 ppm; CHIRL[a] at all levels	Treon and Cleveland (1955)
Rat	Osborne-Mendel	12/M,F	0.5, 2, 10, 50, 100, and 150 ppm	2 yr	Dose-related increase in mortality at 50 ppm and higher; liver/body wt. ratio was increased in males at 10 ppm and above; CHIRL was observed in all treatment groups; hemorrhagic and/or distended urinary bladder associated with nephritis was observed at 100 and 150 ppm; gross enlargement of the liver in all rats fed 50 ppm and above.	Fitzhugh et al. (1964)

(continued on the following page)

Table 8 continued.

TABLE 4-4. (continued)

Species	Strain	No./Sex	Dose	Exposure	Effects	Reference
Rat	Albino	40/M	2 mg/kg/day	6 mo	The RNA content and SGOT and alkaline phosphatase (AP) activities increased 4.38, 2.55, and 5.93 times, respectively, while the bilirubin, cholesterol, and total protein contents decreased 80%, 67%, and 30%, respectively. Histologically, hepatic cells increased 40% in size, while the nuclear and nucleolar size increased 12% and 7%, respectively.	Shakoori et al. (1982)
Dog	Beagle	2/M,F	1, 3 ppm	16 mo	1 female at the 3 ppm had vacuolation of the distal renal tubules; no toxic signs at 1 ppm.	Treon and Cleveland (1955)
Dog	Mongrel	2/M,F	0.2, 0.5, 1, 2, 5, 10 mg/kg/day	25 mo	All dogs at the 1, 2, 5, and 10 mg/kg levels died by the 43rd week; weight loss and convulsions were observed at the higher levels; common lesions observed were fatty degeneration of the liver; fat in the renal tubules; reduced erythroid cells.	Fitzhugh et al. (1964)
Dog	Beagle	5/M,F	0.005, 0.05 mg/kg/day	2 yr	In females at 0.05 mg/kg, liver/body wt. ratio and in both sexes serum alkaline phosphate activity were increased.	Walker et al. (1969)

[a] CHIRL = chlorinated hydrocarbon insecticide rodent liver.

The toxicity of lifetime exposure to dietary levels of 10 ppm dieldrin has been reported in other studies using mice. Survival was decreased in treated CF-1 mice and C3HeB/Fe mice compared to controls at 22 months and 18 months, respectively (Thorpe and Walker, 1973; Davis and Fitzhugh, 1962).

Dogs respond to dieldrin exposure with poor health and increased mortality at dose levels of 0.5 mg/kg body weight/day dieldrin and higher. A dose level of 0.2 mg/kg/day, equivalent to a dietary concentration of 8 ppm, was reported to have no effect on morbidity and mortality (Deichmann et al., 1970). No effect on health, behavior, or body weight gain was observed in beagles (5 dogs/sex/dose group) dosed by capsule with 0, 0.005, or 0.05 mg/kg body weight/day HEOD for two years (Walker et al., 1969). These dose levels were equivalent to 0.1 and 1.0 ppm in the diet.

Liver

The available literature indicates that the liver is the most sensitive target organ to the toxic effects of dieldrin in rodents. A response in the liver was detected at dietary levels as low as 0.1 to 0.5 ppm by researchers at the U.S. FDA (Fitzhugh et al., 1964) and Shell Research Limited (Walker et al., 1969). The effects included increased liver to body weight ratios and hepatic cell changes characteristic of chlorinated insecticide exposure.

Osborne-Mendel rats, 12/sex/dose group, were fed 0, 0.5, 2, 10, 50, 100, and 150 ppm dieldrin in the diet for two years (Fitzhugh et al., 1964). Gross liver enlargement was reported at dose levels greater or equal to 50 ppm. Liver to body weight ratios were significantly increased over ratios in controls at all dose levels in one or both sexes. The data are presented in Table 9.

Table 9. Liver to Body Weight Ratios in Male and Female Osborne-Mendel Rats Fed Dieldrin For Two Years (Fitzhugh et al., 1964).

Dose Level (ppm)	Liver:Body Weight Male Number	Ratio	Female Number	Ratio
0	10	22.94	7	24.55
0.5	11	23.34	8	32.94*
2	11	24.54	11	28.61**
10	7	29.52*	9	30.00**
50	8	27.27**	4	30.16
100	9	38.83*	3	39.85*
150	2	35.31*	0	---

* $p < 0.01$
** $p < 0.05$

Histochemical analysis of hepatic cells revealed enlarged centrilobular hepatic cells with cytoplasmic oxyphilia somewhat increased and peripheral migration of basophilic granules. A dose-related increase in incidence and severity was observed. Table 10 shows the incidence and severity of the hepatic changes by dose level. These data indicate that a LOAEL for microscopic changes in liver cells is 0.5 ppm administered in the diet for a lifetime.

Table 10. Incidence and Severity of Hepatic Cell Changes in Osborne-Mendel Rats Fed Dieldrin for Two Years (Fitzhugh et al., 1964).

Dose Level (ppm)	Histological Description	# Affected Out of # Sectioned
0	Trace to Slight Changes	1 out of 17
0.5	Trace to Slight Changes	5 out of 22
2	Trace to Slight Changes	11 out of 23
10	Trace to Slight Changes	11 out of 18
50	Very Slight to Severe Changes	20 out of 20
100	Very Slight to Severe Changes	18 out of 18
150	Slight to Severe Changes	11 out of 11

Walker et al. (1969) fed 0.1, 1.0, and 10.0 ppm HEOD to Carworth Farm E strain rats, 25/sex/group, for two years. A control group of 45 rats/sex were fed an untreated powder diet for the same time period. An additional 15 rats/sex were fed the control and treated diets and 5 rats/sex/group were killed on weeks 26, 52, and 78 of feeding. Hematological, clinical chemical, and urinary parameters were measured for treatment-related changes. A complete microscopic examination of body tissues was conducted on all animals in the test.

Differences in tissue pathology were noted between the dosed and control groups after two years. Significantly increased liver weight and liver to body weight ratios were observed in female rats fed 1 and 10 ppm HEOD. The increase was dose-related and began in the 0.1 ppm dose group. A slight increase in liver to body weight ratio was also noted in males, but the difference between treated and control rats was not significant. Typical organochlorine insecticide changes were observed only in males and females in the 10 ppm dose group. The data, presented in Table 11, indicate a LOAEL for increased liver weight of 0.1 ppm fed for a life-time, however hepatic cell changes were not detected in these rats until 10 ppm.

Table 11. Liver Weight and Liver to Body Weight Ratios in CFE Strain Rats Fed HEOD for Two Years (Walker et al., 1969).

Dose Level (ppm)	Males		Females	
	#	Liver:Body Wt.	#	Liver:Body Wt.
0	18	4.08 ± 0.147	18	3.66 ± 0.126
0.1	15	4.33 ± 0.173	13	3.72 ± 0.138
1.0	14	4.61 ± 0.198	10	3.70 ± 0.143 ($p<0.01$)
10.0	10	4.84 ± 0.208	9	3.77 ± 0.119 ($p<0.05$)

In an earlier study, Treon and Cleveland (1955) had induced a significantly increased liver to body weight ratio in male Carworth rats fed a dietary level of 2.5 ppm dieldrin at 18 months. The animals, 40 rats/sex/group, fed 0, 2.5, 12.5, or 25 ppm dieldrin for two years, showed no differences in mortality, survival, or weight gain between treated and control rats. The authors also detected microscopic changes in liver cells characteristic of chlorinated hydrocarbon exposure at all dose levels.

The liver cells of albino rats dosed with 5 mg/kg body weight dieldrin every other day for 100 days were vacuolated after 60 days of dosing (Hurkat, 1977). This was accompanied by margination of cytoplasm. No necrosis of liver cells was observed at any point during the study. A dose level of 5 mg/kg dieldrin by gavage for 15 days produced a diffuse liver necrosis and cell vacuolation, membranous glomerulonephritis and renal tubule nephrosis with vacuolated and necrotic cells, and growth retardation in male Wistar strain albino rats.

These effects were mitigated by large doses of ascorbic acid (Bandyopadhyay et al., 1982).

The liver is also the site of dieldrin toxicity in mice maintained on treated diets for the majority of their lifetimes. Hepatic cellular changes similar to those produced in rats were reported in CF-1 mice fed 0.1 to 20 ppm dieldrin for two years (Walker et al., 1972). Larger hepatocytes with cytoplasmic changes in the centrilobular region were observed. These regressed slowly and to a variable extent after treatment ceased. Unfortunately, the occurrence of the hepatic cell response to dieldrin exposure was not discussed in relation to dose.

Wright et al. (1978) studied the biochemical effects of dieldrin exposure in male rhesus monkeys fed 0 ppm (5 animals), 0.01 ppm (4 animals), 0.1 ppm (5 animals), 0.5 ppm (5 animals), 1.0 ppm (4 animals), 1.75 ppm (2 animals), or 5.0 ppm (1 animal) for approximately six years. Total liver protein was significantly increased at 1.75 ppm and was located primarily in liver microsomes. Enzyme assays evaluating liver succinic dehydrogenase activity, glucose-6-phosphatase activity, and alkaline phosphatase activity found no changes related to exposure. On the other hand, microsomal monooxygenase activity indicated by metabolism of chlorfenvinphos by liver homogenates, the specific activity of liver microsomes using chlorfenvinphos, and the level of p-nitroanisole as substrates, and p-hydroxylation of aniline, was increased in monkeys fed 1.75 ppm and 5 ppm dieldrin. Semilog plots of liver protein or specific activity versus dieldrin concentration showed that the increases began to occur at a dieldrin concentration in the liver of 6 to 7 ppm or a dietary concentration of 1 ppm. The concentration of cytochrome P-450 was significantly increased in microsomal fractions in monkeys fed 0.1 ppm and higher. However, a semilog plot did not indicate an increase in enzyme activity in monkeys fed dieldrin at doses lower than 1 ppm. The maximum increase in cytochrome P-450 concentration in the monkey fed 5 ppm dieldrin was twice that measured in the control group. No changes in liver weight, liver DNA, or liver RNA were observed in these monkeys. A dietary threshold of 1 ppm (25 - 30 ug/kg body weight/day) for the induction of the liver microsomal monooxygenase system in monkeys was suggested by the authors.

Beagles, 5 dogs/sex/dose group, were dosed by capsule with 0, 0.005, or 0.05 mg/kg body weight/day HEOD for two years (Walker et al., 1969). These dose levels were equivalent to 0.1 and 1.0 ppm in the diet. The activity of plasma alkaline phosphatase in male and female dogs dosed with 0.05 mg/kg/day did not decrease at the same rate as the other dose groups after 18 weeks of treatment. The activity increased after 30 weeks and then again during the last six weeks of dieldrin treatment, while it continued to decrease in the other dose groups. The difference from the other groups was statistically significant ($p<0.01$). Liver weights and liver to body weight ratios were elevated in female dogs dosed with 0.005 mg/kg/day, and the increase was significant in the highest dose group. No microscopic changes were found associated with dieldrin treatment. Increased liver to body weight ratios ($p<0.02$) were also reported in beagles fed 3 or 1 ppm dieldrin six days per week for at least 15.6 months.

Kidney

Hemorrhagic and/or distended urinary bladders were observed in Osborne-Mendel rats fed 100 ppm or 150 ppm dieldrin in the diet for two years (Fitzhugh et al., 1964). This condition was found mainly in male rats that did not survive the entire two years and was usually associated with considerable nephritis.

Severe grade nephritis was reported in 12 out of the 72 rats fed 50 ppm or greater, in 4 out of the 72 rats fed less than 50 ppm, and in one out of the 24 control rats. In four males fed 50 ppm or greater, severely nephritic kidneys were accompanied by moderate focal calcification. This was regarded as possibly related to dieldrin exposure. Reuber (1980) re-evaluated the kidney effects reported by Fitzhugh et al. Nephritis was more severe and occurred with greater incidence in males. Females were more sensitive to the acute toxicity of dieldrin. Rats died of nephritis before 76 weeks. Reuber emphasized that the occurrence of nephritis may interfere with the development of tumors in rats.

Histopathological changes were reported in the kidneys of Osborne-Mendel rats fed high concentrations of technical dieldrin (Deichmann et al., 1970). A moderately increased glomerular thickening and sclerosis was noted relative to controls with chronic inflammatory infiltration and capillary thickening.

Vacuolation of the distal renal tubule was observed in one out of two beagles fed 3 ppm dieldrin, six days per week, for two years (Treon and Cleveland, 1955). No such effect was noted in control animals.

Kidney effects were not reported in mice (Walker et al., 1972; Thorpe and Walker, 1973; Davis and Fitzhugh, 1962).

Immune System

Acute, sublethal doses of dieldrin have been shown to compromise humoral immune function in mice. The recovery of MHV3 infected mice was slower in a dieldrin exposed group compared to vehicle and unexposed controls. Dieldrin exposed mice also produced less viral antibody. The capacity of peritoneal macrophages from MHV3-resistant female A/J strain mice to restrict the replication of the MHV3 virus after in vitro reinfection was inhibited by the administration of one intraperitoneal dose of dieldrin, equivalent to 0.6 LD50 (36 mg/kg body weight) (Krzystyniak et al., 1986). Dieldrin had no effect on other cellular variables of macrophage function including cell viability, adherence to plastic, generation of superoxide anion, uptake of ^{51}Cr-labelled sheep erythrocytes, and incorporation of [^{3}H]uridine. Therefore, the intrinsic capacity of MHV3-activated macrophages to restrict the virus appeared to be highly sensitive to sublethal dieldrin exposure. The authors concluded that dieldrin may alter macrophage metabolism.

An increased susceptibility of peritoneal macrophages to MHV3 virus infection was demonstrated in C57Bl/6 mice (Krzystyniak et al., 1985). MHV3-infected mice given a single intraperitoneal or peroral dose of 36 mg/kg body weight dieldrin experienced a significant decrease in the mean time of death. Dieldrin exposure also increased *in vitro* MHV3-induced cytopathic effects in these cells.

The effect of dieldrin exposure on the primary humoral response was studied in C57Bl/6 mice (Bernier et al., 1987). Responses to sheep red blood cells (SRBC), an antigen requiring involvement of T-lymphocytes and LPS, and an antigen triggering the immune system without T-cell involvement were evaluated. The mice were given a single i.p. dose of 36 mg/kg body weight HEOD or corn oil. In three independent experiments (5 animals per group per time), the number of IgM antibody producing cells, determined by the splenic plaque forming cell assay, were decreased by almost 60% compared to controls 4 - 7 days after exposure. The reduction was followed by a gradual return to normal values by 24 days after exposure. A marked decrease of anti-SRBC IgM antibody titer in sera, quantified by the ELISA assay, was also observed 7 - 14 days after exposure. Significant inhibition of anti-LPS IgM antibody producing cells was also induced by dieldrin administration 4 - 14 days after exposure followed by a return to normal at 24 days. The anti-SRBC IgG-plaque forming cell response was progressively decreased following dieldrin exposure with a maximum suppression of 82% at 48 days. The time profile of response indicated an effect on the inductive phase of the humoral immune system to thymo-dependent and thymo-independent antigens.

Sublethal doses of dieldrin, 36 mg/kg body weight, given to donor female A/J(H-2^9) mice seven days prior to grafting of their lymphoid cells into B6XA)F1 recipient mice inhibited graft versus host reaction (GVHR) in the recipient mice (Hugo et al., 1988). Splenomegaly and systemic immunosuppression in recipient mice are characteristic features of GVHR. These parameters were not affected in the recipient mice. A significantly lower relative spleen weight was obtained in dieldrin-treated A/J donor mice compared to positive GVHR controls ($p<0.05$). GVHR-induced immunosuppression in recipient mice was inhibited by dieldrin exposure. This inhibition was indicated by a normal production of plaque-forming cells against sheep red blood cells seven days after the induction of GVHR. GVHR-induced suppression of splenocyte proliferation in response to a mitogen-induced stimulation was also inhibited in recipient mice injected with cells from dieldrin-treated A/J donor mice. The authors concluded that the data indicated a marked impairment of T-cell mediated immunity in dieldrin-treated mice seven days after treatment. An impairment of the collaboration processes between T-cell subpopulations involved in the recognition of a foreign antigen was proposed. Cellular cytotoxicity, impairment of the T-cell ratio in lymphoid organs, modification of the T-cell proliferation potential, and modification of thymic subpopulations were excluded as potential factors in the dieldrin-induced suppression.

An impact on the ability of T-cells to recognize foreign antigens was also suggested in another experiment which demonstrated a 45% inhibition of mixed lymphocyte reaction response in vitro by the same dose level of dieldrin (Hugo et al., 1988). The immunosuppression was noted at seven days post-treatment, while no effect was measured at days four, 14, or 24. No significant difference in lymphoid cell content and viability, T-cell ratio, or T-cell mitogen assay were detected at seven days post-treatment.

Endocrine System

Hiddemen and Cornish (1970), in a Society of Toxicology abstract, reported that the intraperitoneal administration of technical dieldrin at 25 mg/kg in corn oil for 1, 2, 3, or 4 days to adult male albino Sprague-Dawley rats induced hyperglycemia and a prolonged elevation in corticosterone levels in blood. The degree of elevation was related to the period of administration. Adrenalectomized rats did not show a hyperglycemic response. Two groups of rats were given an intraperitoneal injection of either 5 mg/kg dieldrin or corn oil for 10 days, and the fasting blood glucose levels in the exposed rats was 25% higher than controls. The authors concluded that dieldrin affects the normal utilization and metabolism of endogenous glucose and causes alterations in the adrenal cortex.

Central Nervous System

The nervous system is the primary target site associated with high level exposure to the chlorinated hydrocarbon insecticides in humans and animals, and the cyclodiene derivatives are among the most toxic. Acute and chronic exposures are characterized by signs of neuronal hyperexcitability, including tremors, myoclonic jerking and convulsions (Joy et al., 1980). Kindling is a phenomenon that involves a progressive increase in convulsive responses as a result of repeated exposure to levels of electrical stimulation or certain chemicals that do not produce convulsions when administered acutely. Dieldrin administration was shown to cause kindling in Sprague-Dawley rats (10 per dose group) dosed with 5, 10, or 15 mg/kg body weight dieldrin intraperitoneally every other day for 19 administrations (Joy et al., 1980; Bowyer et al., 1980). A group of five animals served as controls. A progressive, dose-related increase in seizure severity was observed. Repetitive administration also resulted in progressive increases in the numbers of animals dying during the experiment.

The increased severity of convulsions was not correlated with the concentration of dieldrin in the brain. Groups of animals were killed one hour after each dieldrin administration, and stable peak brain levels were reached by the third or fourth administration after which peak brain levels remained constant for the remainder of the exposure period. On the other hand, seizure severity increased steadily over all 19 exposures. Brain dieldrin concentrations in rats dosed with 10 or 15 mg/kg one hour after dosing on day 1 were 4.1 ± 1.37 and 5.18 ± 1.47 ug/g, respectively. These were higher than the highest brain concentration ever reached during 19 injections of 5 mg/kg dieldrin. Yet, the seizure severities on day one for the two higher dose groups, 1.2 and 1.4, were well below those of the 5 mg/kg group occurring after two or more administrations. Finally, the convulsive threshold for naive rats (rats not exposed repeatedly) (mean, 5.15 ± 1.37 ug/g) was approximately 40% higher than the highest brain concentration attained with the 5 mg/kg dosing schedule. Therefore the increase in seizure severity was attributed to the kindling phenomenon.

Dieldrin exposure showed interactive effects with other kindling stimuli. PTZ kindled rats were cross reactive when exposed to either 2.5 mg/kg or 5 mg/kg dieldrin every other day for 19 administrations. Also, dieldrin exposure (2.5 mg/kg or 5 mg/kg) enhanced the kindling response initiated by electrical stimulation. The acute administration of 5 and 10 mg/kg or 15 mg/kg dieldrin, followed after one week by electrical stimulation of the amygdala to produce kindling resulted in an enhanced response compared to nonexposed controls. These data indicate that acute or chronic exposures to dieldrin alter CNS function to the point that kindling by other means is facilitated. The authors pointed out that dieldrin is capable of evoking enduring changes in brain function even at exposures producing no overt clinical manifestations including changes in behavior. Continual exposure to dieldrin may sensitize an individual to the point where overt, clinical symptoms of exposure become manifest at lower brain concentrations. The changes in CNS excitability may also leave the nervous system more reactive to other, often adversive, stimuli.

Burt (1975) tested learning acquisition in Wistar rats exposed to dieldrin. Male rats (18 exposed) fed dieldrin concentrations of 5 ppm for 120 days made significantly more initial and total errors and required more trials to reach the criterion of success than the 10 control rats learning to negotiate a Lashley III maze. Rats fed 0.5 ppm dieldrin for 60 days (9 animals) did not show significant differences from the control rats (8 animals). Although 0.5 ppm dieldrin apparently resulted in no effect on behavior, this test may not be sensitive to learning deficits induced at lower doses, and the sample size and length of dosing may have decreased the sensitivity of the test. The administration of acute doses of 2.5 and 5.0 mg/kg dieldrin by oral intubation also had deleterious effects in rats tested using DRL operant conditioning, which differentially reinforces low rates of response.

Muscular efficiency, indicated by the time taken to run a standard distance when pulling weights of increasing magnitude, was affected in trained male albino rats (8 animals per dose group) fed 25 ppm (0.125 mg/kg/day) or 50 ppm (0.25 mg/kg/day) of dieldrin for 60 days prior to testing (Khairy, 1960). The running times of control rats were not altered when pulling 5, 40, and 50 gram weights. A significant trend in increasing running time with increasing dose was observed when the rats pulled 50 grams ($p<0.05$). Running times were also significantly increased within the two dieldrin dose groups as the magnitude of the weight increased ($p<0.01$). The authors reported that no significant differences between the dose groups on body weight or food intake were observed.

Conditioned avoidance response, a test of learning ability, was not affected in the offspring of pregnant albino mice subjected to a subchronic dosing of aldrin (Al-Hachim, 1971). Oral doses of 2 mg/kg or 4 mg/kg aldrin for seven days to pregnant albino mice (7 per group) during the third trimester caused no differences in the number of conditioned avoidance responses out of 16 trials per day compared to the control mice dosed with only corn oil. Three groups of 10 weanlings were tested for seven days. On the other hand, electroshock seizure threshold was significantly increased in the treated groups tested at 38 days of age ($p<0.005$) and body weights of the offspring were significantly decreased ($p<0.005$).

Dieldrin and the subjection of rats to uncontrollable footshock stress had a combined effect on the subsequent performance in a shuttlebox shock escape test (Carlson and Rosellini, 1987). Sprague-Dawley albino rats were evaluated in one of two experiments. In the first experiment, four animals per group received 0, 1.5, or 4.5 mg/kg dieldrin in corn oil by oral gavage. Thirty minutes following treatment, the rats were given 40 five second 1.0 mA footshock trials on a variable time schedule of 60 seconds. A shuttle box shock escape test was conducted eight hours later. After five initial learning trials, the rats were given 60 1.0 mA grid shock trials where the rat had to cross from one side to the other and then back to terminate the shock. Dieldrin exposure produced a dose-related increase in mean escape latency and in the number of shock escape failures. Escape latency in both exposure groups was significantly longer than controls in all six 10-trial blocks.

The procedure in the second experiment consisted of stressing pairs of rats by giving one rat 40 controllable shocks (10 terminated by a one bar press response and 30 terminated by a two bar press response) while at the same time its pairmate received an identical shock which was terminated only by the actions of its partner. A third group was confined in an operant chamber for a similar time period but was not shocked. Animals in the three different stressor groups were given 0, 0.5, 1.5, or 4.5 mg/kg dieldrin 30 minutes before the stress treatment. Eight hours later, shuttlebox escape testing was conducted.

A dose-related effect on performance was observed in the group of rats stressed by inescapable shock (p= 0.007). The rats stressed with the controllable shocks demonstrated a decreased latency during the first 10 one bar press trials which disappeared during the 30 two bar press trials. There was a significant effect on latency between stressor groups (0<0.001) and a significant group by dose interaction ($p<0.001$). Within the group stressed by inescapable shock, rats dosed 4.5 mg/kg took significantly longer to escape shock than rats given 1.5 mg/kg, and both of the higher dose groups were significantly different from the 0.5 and vehicle dosed groups. Animals given inescapable shock and 1.5 or 4.5 mg/kg dieldrin also differed significantly from rats given controllable shock or no shocks. The authors concluded that prior exposure to uncontrollable stress can alter a subject's susceptibility to the functional consequences of low acute doses of dieldrin.

Dieldrin exposure caused deficits in learning acquisition, but did not affect the performance of a learned task in squirrel monkeys orally exposed to 0.1 mg/kg/day dieldrin for 55 days (Smith et al., 1976). A nonspatial successive discrimination reversal task, chosen for its prolonged learning acquisition component, combined with a spatial alternation component was used. The test consisted of a training period where the monkey learned to complete a specified number of consecutive correct responses, with one stimulus designated as correct. Then the previously correct stimulus became incorrect and vice versa. Each monkey (12 animals) had one daily half hour session and the number of reversals attained each day was counted. The dieldrin exposure began on the tenth day of training. Technical dieldrin, 0.1 or 0.01 mg/kg, injected into a marshmallow was fed after the daily sessions. Control monkeys received a marshmallow injected with the vehicle, ethanol. During the 55 day exposure period, monkeys in the high dose group learned the task more slowly ($p<0.015$) and attained fewer reversals per session than controls ($p<0.001$). The low dose group learned the task at a rate similar to the control group. Testing was continued for an additional 54 days during which the monkeys in the low dose group were fed the high dose of 0.1 mg/kg and the monkeys in the high dose were fed the control diet. The change in dose did not alter the performance in each group established by the end of the 55 day training period. The authors concluded that 0.1 mg/kg/day produced severely retarded successive discrimination reversal learning, but not the performance of a learned task. It was suggested that levels below 0.1 mg/kg/day may also disrupt learning, particularly if dosing was carried out several months prior to the behavioral sequence.

CONCLUSION

Chemical intoxication, including convulsions, has occurred in humans exposed to high levels of dieldrin occupationally (Van Raalte, 1977) and through poisoning incidents (U.S. EPA, 1987). A no effect level for dieldrin intoxication was established at 200 ppb in blood, equivalent to an oral intake of 33 ug/kg/day (Van Raalte, 1977). Various physical and chemical parameters were not different from controls among 233 dieldrin exposed workers with four to 15 years of exposure (Van Raalte, 1977). Tests of hepatic enzyme induction were also not altered in workers with the highest blood levels of dieldrin. Plasma alkaline phosphatase activity was not elevated in individuals intentionally dosed with up to 211 ug HEOD per day or 3 ug/kg/day for two years (Hunter and Robinson, 1967; Hunter et al., 1969). Six individuals who had eaten contaminated eggs and poultry and handled contaminated litter for approximately four months were found to have raised alkaline phosphatase levels. Blood dieldrin concentrations in this group ranged from 0.003 to 0.011 ug/ml (3 to 11 ppb).

Rats are the most sensitive species to the toxic effects of dieldrin administration on the liver. A dose of 0.5 ppm dieldrin in the diets of

Osborne-Mendel rats for two years, equivalent to 0.025 mg/kg body weight/day, produced increased liver to body weight ratios and pathological changes in the hepatic cells of females (Fitzhugh et al., 1964). These effects were dose-related in males and females. The same effect was noted in other rat strains between one and ten ppm dieldrin (Walker et al., 1969; Treon and Cleveland, 1955).

Other species also show a hepatic response to dieldrin exposure. Hepatic cellular changes similar to those produced in rats were reported in mice by Walker et al. (1972). Microsomal mono-oxygenase activity was increased in rhesus monkeys fed 1.75 ppm and 5 ppm dieldrin for six years (Wright et al., 1978). A semilog plot of specific activity versus dieldrin concentration indicated a dietary threshold of 1.0 ppm, equivalent to 0.025 mg/kg body weight/day. Plasma alkaline phosphatase activity was elevated relative to controls in male and female beagles dosed with 0.05 mg/kg body weight/day for two years (Walker et al., 1969). Liver weights and liver to body weight ratios were significantly elevated in female dogs in this group, however, no microscopic changes were found in liver cells associated with dieldrin treatment.

An effect on survival and morbidity was observed in Osborne-Mendel rats fed 30 and 50 ppm or more dieldrin for two years (Fitzhugh et al., 1964; Deichmann et al., 1970). Irritability, tremors and occasional convulsions were reported in CFE rats fed 10 ppm dieldrin for two years, but mortality, body weight, food intake, and general health were not affected (Walker et al., 1969). The lifespans of male and female CF-1 mice were shortened by the consumption of 10 and 20 ppm dieldrin in their diets for two years (Walker et al., 1972; Thorpe and Walker, 1973; Davis and Fitzhugh, 1962). Levels of 0.5 mg/kg/day dieldrin increased mortality in dogs while no effect on mortality or morbidity was reported at 0.2 mg/kg/day.

Learning acquisition was adversely affected in Wistar rats fed dieldrin concentrations of 5 ppm for 120 days and tested in a Lashley III maze (Burt, 1975). A dose of 0.5 ppm did not affect learning in this test, although sample sizes were small. Conditioned avoidance response was not affected in offspring of pregnant albino mice given oral doses of 2 mg/kg or 4 mg/kg aldrin for seven days during the third trimester, although electroshock seizure threshold was significantly increased in treated groups (Al-Hachim, 1971). An interaction between uncontrollable stress and acute dieldrin exposure (1.5 mg/kg or 4.5 mg/kg) was demonstrated in rats evaluated in a more complicated shock avoidance test (Carlson and Rosellini, 1987). Learning acquisition was compromised in squirrel monkeys exposed orally to 0.1 mg/kg/day for 55 days during a behavioral test. A dose of 0.01 mg/kg/day did not change learning rates (Smith et al., 1976). More research is needed on the effect of dieldrin exposure on learning using a variety of behavioral tests, larger sample sizes, lower dose levels, and longer exposure periods.

Higher dietary dieldrin concentrations have also been associated with effects on the kidney, a depression of T-lymphocyte mediated and other immune responses, alterations in the endocrine system, and enhanced CNS excitability resulting in a kindling response. There is no literature currently available on the adverse effects of chronic, low level dieldrin exposure on the immune response, behavioral/CNS function, or the endocrine system in experimental animals.

THE EFFECT OF DIELDRIN EXPOSURE ON REPRODUCTION

Reproductive effects in human populations have not been investigated in relation to dieldrin exposure. Exposure to dieldrin results in embryotoxicity, fetotoxicity, and decreased pup viability in mice and rats. These effects were induced at moderate dose levels, which do not result in maternal mortality, but do cause hepatic cell changes in dams. Abnormal reproduction has also been reported in dieldrin-exposed dogs and rabbits.

Fertility

Wistar rats, ten females per dose group, were bred at 146 days of age and again at 336 days of age after 118 days and 308 days on dieldrin treated diets (Harr et al., 1970). Fertility was reduced in dieldrin treated rats; 73% of 146 day old treated females and 33% of 336 day old treated females became pregnant. In the control group, 90% and 50% of 146 day olds and 33 day olds, respectively, conceived. Conception among 146 day old dams fed 2.5 ppm and 5.0 ppm dieldrin was 90% and 80%, respectively. Conception among 336 day old dams was 50% and 33%, respectively. Higher dose levels were complicated by mortality. Sample size was relatively low, and the statistical significance of the fertility data was not assessed. Therefore, it is difficult to determine whether or not dieldrin treatment caused a reduction in conception in the rats.

Fertility was affected in Carworth rats fed dietary levels of 2.5, 12.5 or 25 ppm dieldrin over three generations. An early decrease in the number of pregnant rats relative to controls was observed, but the decrease was reported to disappear in later generations (Treon and Cleveland, 1955). No reduction in the number of offspring per litter was observed. This study cannot be evaluated because no data was presented regarding the number of females mated, the indices of fertility, embryo or fetal toxicity, or pup viability.

An effect on fertility in first generation Swiss white mice was noted in a six generation feeding study using dietary concentrations of 3, 10, and 25 ppm dieldrin (Keplinger et al., 1970). Each group contained 4 males and 14 females, 120 days old, and two litters were obtained from each generation. Very marked effects occurred in the 25 ppm dieldrin group. Only two females delivered a first litter compared to 12 to 14 females in the other treated groups and controls. No females delivered a second litter. Dieldrin at 10 ppm in the diet caused a significantly decreased fertility index in first generation females after a second mating period. Fertility was not adversely affected after the first mating. Feeding at 10 ppm dieldrin was not continued after the parental generation. A dietary concentration of 3 ppm did not decrease the fertility of the treated female mice. It is not possible to conclude that 3 ppm dieldrin would result in no effect because relatively small sample sizes were used.

Marked effects on the fertility of Swiss mice in the parental and second generations fed 25 ppm dieldrin were reported by Deichmann et al. (1972). Reduced fertility was also observed in mice fed 3 and 10 ppm dieldrin.

Fertility and fecundity were not affected in CFW strain Swiss mice fed 5 ppm technical grade dieldrin for 30 days prior to mating and continued for 90 days after the pairing of treated male and female mice (Good and Ware, 1969).

Subnormal reproduction was induced in beagle dogs dosed with aldrin for 14 months (Deichmann et al., 1971). Eleven beagle dogs were given 0.15 mg/kg (4 females) and 0.3 mg/kg (4 females and 3 males) aldrin by capsule five days per week. The dogs were bred after dosing with aldrin was discontinued. The estrous cycle in females was delayed by 7 to 12 months, and mammary development and milk production were reported to be severely depressed. Stillbirth occurred in one female dosed with 0.15 mg/kg and in two females dosed with 0.3 mg/kg. Two females dosed with 0.15 mg/kg aldrin did not come into heat during eight months after dosing with aldrin was discontinued.

Embryotoxicity/Fetotoxicity

Good and Ware (1969) observed reduced litter size in male and female CFW strain Swiss mice fed 0 or 5 ppm technical grade dieldrin for 30 days prior to mating. Each test group, composed of 101 pairs, was continued on the diets for 90 days. The percent pairs with mortality in the treated group (5.94%) was not significantly different from the control group (6.93%). The size of all litters produced by treated pairs was smaller (8.74 ± 0.14) than controls (9.2 ± 0.15; $p<0.05$). The time required to produce the litters was not different suggesting no effect on the estrus cycle.

SWV strain mice who had previously raised a litter were maintained on experimental diets for four weeks and were then mated with males for a two week period during which time the males ate the same treated diet as the females (Virgo and Bellward, 1975). The animals, 18 or 19 per dose group, were fed 0, 2.5, 5, 10, 15, 20, or 25 ppm technical dieldrin in their diets. Litters were kept with the females until weaning age.

Dose levels of 20 and 25 ppm caused high maternal mortality. After four weeks on the treated diets, 8 out of 18 mice fed 20 ppm and 13 out of 18 mice fed 25 ppm dieldrin had died compared to no deaths in the other groups. Mating was not affected by treatment. All bred females became pregnant except in the mice fed 10 or 15 ppm dieldrin, 18% of whom did not. Ovulation and implantation were evaluated in an additional set of mice (30 mice per group). In the mice fed 15 ppm dieldrin, 33% (14 out of 21) of pregnant mice were found to have no implants while 7% (14 out of 15) of pregnant control mice lacked implants. The difference had marginal statistical significance ($p=0.061$). The authors concluded that the effect of dieldrin on fertility was slight but probably real and appeared to occur prior to implantation but after ovulation. Of the females lacking implantation sites, 5 had corpora lutea and only two did not. All females fed 25 ppm dieldrin had implantations. However, high mortality occurred in this group perhaps masking embryotoxic effects.

Litter size decreased slightly as the concentration of dieldrin in the diet increased. The decrease was still small but statistically significant in the highest dose group. The mean number of pups per litter was 11 ± 0.63 in the treated mice compared to 13.2 ± 1.06 in controls. Fetal mortality, indicated by the mean number of placental scars minus the mean litter size divided by the mean number of placental scars, was highest in the control mice. Fetal mortality was 18% in controls, 17.7% in the 2.5 ppm group, and ranged from 2.5% to 7.6% in the other treatment groups. However, placental scaring occurs only after day 10 of gestation. Therefore, the cause of the decreased litter size was judged to occur prior to implantation. This was in agreement with a finding of a reduction of the number of implantation sites in females fed 25 ppm dieldrin. Among mice with implantation sites, mice fed 25 ppm had 11.1 ± 0.69 pups/litter ($p<0.05$), mice fed 15 ppm had 11.5 ± 0.55 pups/litter, and control mice had 12.5 ± 0.35 pups/litter.

Pregnant albino CD-1 strain mice and CD strain rats were dosed with 1.5, 3.0, or 6.0 mg/kg/day dieldrin by gastric intubation during the period of organogenesis on days 7 - 16 of gestation (Chernoff et al., 1975). The mice and rats were killed on day 18 and 21, respectively.

Weight gain in mice dosed with 6 mg/kg/day was significantly less than controls ($p<0.05$). The average weight gain in the highest dose group was 0.3 ± 2.1 g compared to 2.8 ± 2.3 g in controls. Liver/body weight ratios were significantly increased in a dose-related manner in the treated groups ($p<0.01$). The average liver/body weight ratios in the control and three treatment groups were 7.3 ± 0.7, 8.4 ± 0.6 (1.5 mg/kg/day), 9.1 ± 1.2 (3.0 mg/kg/day, $p<0.05$), and 10.7 ± 0.8 (6.0 mg/kg/day, $p<0.05$), respectively. A significantly increased ($p<0.05$) percentage of supernumerary ribs at the two highest doses was observed in mice whose fetuses weighed an average of 1.28 g. A significant ($p<0.05$) decrease in the number of caudal ossification centers was found at the highest dose in these fetuses as well as those weighing an average of 0.94 g. These anomalies were considered to be signs of fetotoxicity.

Rats in the highest dose group had a 41% mortality rate. Average weight gain was also significantly decreased compared to controls (55.7 ± 17.5 g versus 82.2 ± 16.7 g; $p<0.01$). The fetuses of dieldrin treated rats showed no significant differences from the controls in mortality, weight, or occurrence of anomalies. There were no significant differences in the average number of sternal or caudal ossification centers.

Teratogenicity

Ottolenghi et al.(1973) gave pregnant CD-1 mice or Syrian golden hamsters a single dose of 15 mg/kg or 30 mg/kg dieldrin on day 9 of gestation for mice, and on day 7, 8, or 9 of gestation for hamsters. The animals were killed and examined on day 18 (mice) or day 14 (hamsters).

Fetal survival or fetal weight in treated mice were not different from control mice. Webbed foot and cleft palate were observed in the treated group; webbed foot in 6.4 fetuses per litter (8%) and cleft palate in 14.9 fetuses per litter (13%). These anomalies were not reported in the control group.

Fetal deaths among hamsters treated on days 7, 8, or 9 were significantly higher than controls. Fetal mortality among hamsters treated on days 7, 8, or 9 was 20.4%, 25.4%, and 23.4%, respectively, compared to 10.3% in hamsters dosed only with corn oil or 2.6% in untreated controls. Fetal body weights in hamsters dosed at each gestation day were also significantly lower than controls. The weights of fetuses exposed on days 7, 8, or 9 were 0.747 ± 0.135 g, 0.703 ± 0.128 g, and 0.726 ± 0.071 g, respectively, compared to 0.939 ± 0.055 g, 0.907 ± 0.044 g, and 0.955 ± 0.044 g in controls treated only with corn oil on the same gestation days. Anomalies were most prevalent in hamsters treated on day 8 when incidence was 24.6% open eye ($p<0.01$), 15.9% webbed foot, and 16.1% cleft palate compared to 0.4% open eye, 0.0% webbed foot, and 0.3% cleft palate in controls dosed with corn oil on days 7, 8, or 9 of gestation.

Dix et al. (1977) evaluated the effects of dieldrin exposure on reproduction in CF-1 mice using two different dosing vehicles. In one experiment virgin female mice (7 weeks old) were dosed with 1.5 or 4.0 mg/kg/day HEOD in corn oil by oral intubation on days 6 through 14 of gestation. The pregnancy rate was low in all mice. The second experiment was conducted using 10 week old virgin females and males of proven fertility. Females were given 0.25 or 0.5 mg/kg/day HEOD in dimethyl sulphoxide (DMSO) for the same period during gestation. Control groups were administered the respective vehicle or were sham dosed. Pregnant females were killed on gestation day 18 and fetuses were examined for abnormalities.

HEOD in corn oil did not alter the number of litters produced compared to controls, or alter the relative number of males and females in the litters compared to control litters. Mean litter size, resorptions, or fetal deaths were similar to controls as were mean live fetal weights. An increase in the incidence of delayed ossification among fetuses exposed to a maternal dose of 4 mg/kg/day HEOD in corn oil was the only significant effect.

HEOD in DMSO caused toxicity and mortality among treated females. These effects were also observed in mice dosed with the vehicle, DMSO, alone. The number of pregnant mice surviving to term in the 0.5 and 1.0 mg/kg/day HEOD in DMSO groups were significantly lower than either of the control groups. Reduced body weight gain was also observed in mice treated with HEOD in DMSO or the vehicle alone. Although no differences between treated and control mice were noted with regard to the parameters of fetotoxicity, mice exposed to HEOD in DMSO or DMSO alone had significantly lower fetal body weights when compared to the nontreated controls. An increased incidence of delayed ossification was observed in litters exposed to maternal doses of 0.5 mg/kg/day HEOD in DMSO and in the DMSO control litters compared to nontreated control mice. A dose response relationship was not apparent. DMSO was associated with toxicity in the mice, and the authors concluded that DMSO may have been responsible for some of the toxic effects seen in the experiment.

Although some minor skeletal anomalies indicative of retarded development were induced in the 0.5 mg/kg/day HEOD mice and in the DMSO vehicle group, no significant increases in structural abnormalities occurred in either experiment. Therefore, the authors concluded that no teratologic effects associated with dieldrin exposure were induced in the mice.

Viability of Offspring

Treon and Cleveland (1955) reported increased mortality among nursing Carworth rat offspring that was severe in groups fed 12.5 and 25 ppm was and slight to moderate in rats fed 2.5 ppm dieldrin. The pups showed no difference in weight gain from control rats. Unfortunately the data were not presented, and certain details of study design were not described making it difficult to evaluate these results.

Keplinger et al. (1970) also reported marked mortality in pups (Swiss white rats) exposed to maternal doses of 10 ppm dieldrin in the diet. Only 67% of the first litter pups of the parental generation parents fed 10 ppm dieldrin were alive at four days and 31% of the pups were alive at four months. Of the first litter pups in the 3 ppm dieldrin exposure group, 98% were alive at four days and 92% were alive at four months. Survival in control pups was 94% at four days and 73% at four months. Mortality was more pronounced in the second litter pups of first generation parents fed 10 ppm dieldrin. Only 14 out of 74 pups born alive in this dose group were alive at four months, and a second generation was not attempted. Survival remained high in pups born to parents fed 3 ppm dieldrin in the diet over six generations except for a significantly decreased lactation index in either the first or second litter pups of the second and third generation. The lactation index is a measure of survival to weaning. Pathological changes were observed, primarily in the livers of mice fed either 3 ppm or 10 ppm dieldrin, however, no increase in liver weight was noted.

Harr et al. (1970) studied the reproductive effects of dieldrin exposure in rats fed very low dietary concentrations. Wistar rats, 10 females per dose, were bred at 146 days of age and again at 336 days of age after 118 and 308 days on dieldrin treated diets. Survival to weaning age of offspring in the first litters exposed to maternal doses of 0.31, 0.63, or 1.25 ppm was 90%, 97%, and 100%, respectively. The survival of second litter pups in the same dose groups was 100%, 53%, and 100%. Survival in control pups was 100%. Although an effect on pup viability was apparent at 0.63 ppm, the response was not dose-related.

Survival in first litter pups in the 2.5, 5.0, and 10 ppm dose groups was 60%, 83%, and 26%, respectively. In second litter pups, survival to weaning was 82%, 0% and 0%. Conception and litter size were also severely affected in the 5.0 ppm and 10.0 ppm females bred a second time. The pups died either from starvation during the first five days or from convulsive seizures at 5 - 10 days of age. No statistical evaluation of the data was reported, however the authors calculated that the highest dietary dieldrin concentration consistent with normal reproduction was 0.24 ppm.

Female rats fed 0.31 - 1.25 ppm were reported to have pathologic changes in the liver including centrilobular degeneration and peripheral hyperplasia, swollen hepatic cells with foamy eosinophilic cytoplasm and pyknotic nuclei. Vascular and encephalitic lesions were also reported. Hepatic degeneration was common in the pups of these rats. Pups from dams fed 0.08 - 0.16 ppm dieldrin developed cerebral edema and external hydrocephalus. Focal polioencephalomalacia was reported in the cerebrum and cerebellum. Reproductive parameters were not different from controls.

Deichmann et al. (1972) reported marked effects on gestation, viability, lactation or survival in the offspring of parental and second generation Swiss mice fed 25 ppm dieldrin. Less marked but significant effects also occurred in the first and second generation offspring exposed to maternal doses of 3 and 10 ppm dieldrin.

Dieldrin decreased the number of litters in which pups of Swiss-Vancouver mice were weaned. This was considered by the authors to be the chemical's most important effect (Virgo and Bellward, 1975). All pups were dead four days post-partum in groups of mothers fed 10, 15, 20, or 25 ppm technical dieldrin in their diets four weeks prior to mating and throughout gestation and nursing of offspring. Sixty-nine percent of control dams weaned pups compared to 20% of dams fed 5 ppm dieldrin and 53% of dams fed 2.5 ppm dieldrin. The reduction was statistically significant in the 5 ppm dose group. There was no increase in pup mortality within the litters that were successfully raised to weaning. Therefore, dieldrin caused pup mortality in an all or none manner within each litter.

Pup killing due to abnormal maternal behavior was the most important proximate cause of mortality at dose levels of 10 ppm dieldrin or higher. Weaning age pups in the 2.5 and 5 ppm dose groups weighed 12.1 ± 1.4 grams and 10.4 ± 0.6 grams, respectively, which was not significantly different from control pup weights, 9.7 ± 1.6 grams. Pups in the dieldrin exposed litters that did not survive to weaning weighed 3 - 13% less at birth than the control pups, and the decrease was significant in the 25 ppm dose group. In those litters in the 2.5 and 5.0 ppm groups that did not survive, the pups were inactive and listless, milk content was reduced or absent, and they failed to grow before death. The authors suggested that the pups were congenitally inviable. Dams in the two lower dose groups that successfully raised litters to weaning delivered pups with weights the same as controls (1.15 grams). Liver weights in dams correlated with litter loss.

Costella and Virgo (1980) studied the causes of inviability in dieldrin exposed pups (CD-1 strain mice). Treated pups were found to be hypoglycemic at delivery (75% of normal) and remained so until death. There was no evidence that dieldrin impaired either glycogenolysis or gluconeogenesis.

Conclusion

Despite the existence of few adequately reported studies on reproductive effects, exposure to dieldrin results in embryotoxicity, fetotoxicity, and decreased pup viability in mice and rats. These effects were induced at moderate dose levels, which do not result in maternal mortality, but do cause hepatic changes in dams. Pup viability was reduced in exposed rats at 2.5 ppm dieldrin and higher. Abnormal reproduction has also been reported in dieldrin-exposed dogs and rabbits.

Fertility was adversely affected in Swiss white rats fed 10 ppm dieldrin or more over six generations (Keplinger et al., 1970). Although no effect on fertility was observed in rats fed 3 ppm, a NOAEL cannot be determined because sample sizes used in the study were relatively small. Statistical analysis of the data was conducted, however, and the study was adequate for a weight of evidence determination. Delayed estrus cycles were produced in female beagles dosed with 0.15 mg/kg and 0.3 mg/kg aldrin by capsule five days per week for

14 months (Deichmann et al., 1971). Two females did not come into heat during eight months after dosing was discontinued. The reproductive effects were quite severe in the dogs at these dose levels. The results were presented as case studies of individual dogs and no statistical analysis was conducted.

Litter size was reduced in Swiss white mice fed 10 ppm dieldrin or more over six generations (Keplinger et al., 1970). Virgo and Bellward (1975) also reported decreased litter size at this dose level and attributed it to an effect on implantation. A significant decrease in litter size was reported in CFW strain Swiss mice fed 5 ppm dieldrin for 30 days prior to mating (Good and Ware, 1969). Developmental effects including an increase in supernumerary ribs and delayed ossification were also induced in mice at higher dieldrin exposures (Chernoff et al., 1975; Dix et al., 1977). The studies by Virgo and Bellward (1975) and Good and Ware (1969) are adequate to contribute to a weight of evidence determination for developmental toxicity.

A reduction in pup survival to weaning was observed in Wistar rats fed dietary concentrations of 2.5 ppm dieldrin or more 118 days prior to mating and through the weaning of litters (Harr et al., 1970). The data were not statistically evaluated and sample sizes were relatively small (10 rats/dose). In addition, the data were not presented by litter. Although reproductive parameters were no different from controls in mice fed 0.08 - 0.16 ppm, pups were found to have abnormal pathology including cerebral edema, external hydrocephalus, and focal polioencephalomalacia. The significance of these effects, not reported by other investigators, should be pursued further as they occurred at a very low exposure level.

Pup viability was significantly reduced in Swiss-Vancouver mice fed 5 ppm dieldrin four weeks prior to mating and throughout gestation and nursing (Virgo and Bellward, 1975). The survival of pups to weaning was also reduced (not significant) in the 2.5 ppm group. Dieldrin caused pup mortality in an all or none manner within each litter. At these dose levels, the pups in the litters that did not survive were considered to be congenitally inviable. This study appears to have been adequately conducted and was thoroughly reported. A relatively large number of mice per dose level were used (18 - 19) and the data were evaluated statistically with the dam as the statistical unit.

A weight of evidence determination of Probable Positive Reproductive II for dieldrin is made based on a significantly decreased fertility index in Swiss white rats fed 10 ppm dieldrin (Keplinger et al., 1970). Dieldrin is classified as a Probable Positive Developmental toxin based upon the effects on litter size and pup viability reported by Virgo and Bellward (1975) and Good and Ware (1969).

THE CARCINOGENICITY OF DIELDRIN

Studies in humans, reviewed in this report, are inadequate to make a determination regarding the carcinogenic potential of dieldrin. The experimental evidence of carcinogenicity in animals has been reviewed by the U.S. EPA (1987) and is summarized in this section.

EPIDEMIOLOGICAL EVIDENCE

Organochlorine pesticide concentrations were determined between January 1964 and June 1967 in autopsy tissues of 271 patients in a Florida hospital previously exhibiting liver, brain, and other neurological disease. These were compared with a group of randomly selected autopsy cases and a group labelled infectious diseases (Radomski et al., 1968). Whenever possible, information was attained from the next of kin regarding demographic variables, the illness, and potential exposure to pesticides. Individuals with portal cirrhosis (33 cases) had mean levels of dieldrin in body fat and liver tissue that were significantly higher than those of the normal population ($p<0.001$). Concentrations were 0.5 ± 0.36 ppm in the fat of those with portal cirrhosis and 0.21 ± 0.15 ppm in the fat of normal individuals (42 cases). Dieldrin levels were normal in cases with fatty metamorphosis of the liver (14 cases) or metastatic malignancy of the liver (30 cases). The 24 patients dying of encephalomacia had significantly elevated dieldrin levels in fat tissue (0.5 ± 0.33 ppm, $p<0.01$) compared to normal patients (0.21 ± 0.15 ppm). Brain concentrations, however, were slightly lower (0.01 ± 0.01 ppm versus 0.04 ± 0.01 ppm). The same pattern was apparent in cases with old or recent evidence of cerebral hemorrhage. Eight cases with hypertension consistently had dieldrin concentrations in fat (mean 0.73 ± 0.59 ppm) that were two to four times those of controls (mean 0.21 ± 0.15 ppm). Patients with carcinoma (40 cases), including carcinoma of the lungs, stomach, rectum, pancreas, prostate and bladder, also had elevated dieldrin concentrations in fat compared to controls (0.55 ± 0.34 versus 0.21 ± 0.15 ppm). Fat concentrations were found to be correlated with pesticide levels in the brain. No correlation was observed between the fat and liver concentrations measured.

The mortality of insecticide workers employed at a plant of Shell Nederland Chemie B.V. at Pernis-Rotterdam which has manufactured and formulated aldrin and dieldrin since 1954 has been monitored since the late 1960's (Versteeg and Jager, 1973; Van Raalte, 1977; Ribbens, 1985). Endrin had also been manufactured at the plant and, for a short time, Telodrin. Vital status and causes of death were obtained for 232 of a group of 233 workers from municipal register offices, the Social Insurance Bank, the Central Bureau of Genealogy, the Government Inspectorate of Population Registers, and the Dutch Shell Pension Fund. The cause of death was obtained from the physician who had signed the death certificate when possible, or from the Central Bureau of Statistics. By the end of the study, December 31, 1982, the exposed group had been exposed for a mean of 11 years (range 4-27 years) and were followed for a mean of 24 years (range 4-29 years).

Age specific mortality was compared to deaths in the male Dutch population. A total of 25 deaths had occurred in the cohort compared to 38 expected deaths. A total of nine cancer deaths were observed compared to 12 expected (SMR = 0.75; 95% confidence limits: 0.25 - 1.25). The cancer sites involved were three cases of lung cancer and one case each of cancer of the stomach, pancreas, bladder, kidney, multiple myeloma, and cerebral glioma. The authors reported no correlation with duration of exposure or observation period. The mean dieldrin blood concentrations in early years had been 69 ug/l in 1964 to 24.7 ug/l in 1969.

Several limitations of the study preclude a definitive conclusion regarding the carcinogenic potential of dieldrin exposure in this population. Although high exposure levels were evident in early years indicated by clinical signs and symptoms of intoxication, the exposure status of the 232 workers was not described in detail. The period of follow-up was probably adequate for the development of cancer, however the size of the cohort was small with very few deaths by the end of the study. Finally, no control group was followed. A control group would have been a better comparison group than the national male Dutch population.

Mortality in another cohort employed at an organochlorine pesticide manufacturing plant in Colorado has been studied (Ditraglia et al., 1981). Aldrin and dieldrin were manufactured from 1946 to the 1970's. Endrin was produced from 1953 to 1965. Organobromine and organophosphate pesticides were also manufactured. Workers were also exposed to numerous chemical precursors. Workers who had achieved at least six months' employment prior to December 31, 1964 were included in the study. Vital status as of December 31, 1976 was ascertained from federal and state agencies, including the Social Security Administration and state motor vehicle offices, the U.S. Postal Mail Correction Services and other methods. The causes of death were coded by a nosologist from death certificates according to the ICDA in effect at the time of death. The cohort was comprised of 1155 workers and 24,939 person-years of observation. Vital status for 10% of the cohort was not determined.

Fifteen percent (173 workers) of the cohort were deceased resulting in a SMR of 84 (95% confidence limits: 72 - 98). Thirty-one deaths from cancer were observed resulting in a SMR of 82 (95% confidence limits: 56 - 116). The SMRs for several specific cancer sites were elevated, but not statistically significant, including cancer of the esophagus (SMR = 235), rectum (SMR = 242), liver (SMR = 225), and lymphatic and hematopoietic system (SMR = 147). A significant excess of nonmalignant respiratory system disease was also observed among workers at the plant. (22 observed versus 10.4 expected; SMR = 212, $p < 0.01$). The excess was accounted for by pneumonia and other respiratory diseases.

This study was also inadequate to draw conclusions regarding the carcinogenic potential of dieldrin in the workers. The inability to determine vital status for 10% of the cohort, and certain aspects of study design may have resulted in an underestimate of the risk. The investigators assumed that all those with an unknown vital status were alive as of the cut-off date. This potential overestimate of the number of person years at risk would have resulted in an inflated number of expected deaths. The potentially underestimated number of observed deaths and the overestimate of expected deaths could have combined to produce an underestimate of the actual risk. The demographic characteristics of the sample population were not described. In addition, the degree of dieldrin exposure within the cohort was not described, and the individual effects of the various chemicals to which the workers were exposed could not be determined. Although age-specific expected mortality was calculated based on the U.S. white male cause-specific mortality rates, sex and race were not included in the analysis, and other confounding variables were not evaluated. Although the length of follow-up (greater than

25 years) was probably adequate, the number of deaths by the end of the study was low.

EVIDENCE IN EXPERIMENTAL ANIMALS

The carcinogenicity of dieldrin in experimental animals was evaluated by the U.S. Environmental Protection Agency in 1987 based on the available literature published through 1985. The literature was thoroughly described, and no new bioassays involving dieldrin exposure have been conducted since this review was written. Two tables from the U.S. EPA review (Tables 12 and 13) that present the study designs and effects observed by the carcinogenesis bioassays of dieldrin in mice and rats are reproduced in this report. A conclusion regarding the adequacy of the study to allow a determination of carcinogenic potential is also included in the tables. A summary of the effects observed in experimental animals is provided in this report, and the reader is referred to the U.S. EPA assessment (1987) for the details on individual tests.

Adequate studies have been conducted in mice and rats. The dietary administration of dieldrin has been unambiguously associated with the development of benign and malignant liver tumors in various strains of mice. Dieldrin exposure is not associated with cancer in rats or hamsters. Tests have also been conducted in dogs and monkeys, however, they were not adequate to make a determination regarding their carcinogenic potential.

Mice

Several bioassays have been conducted using mice (Table 12). Lifetime exposure to dieldrin in the diet has produced tumors in the following strains; C3HeB/Fe mice (Davis and Fitzhugh, 1962), C3H mice (Davis, 1965), Swiss-Webster mice (MacDonald et al., 1972), CF-1 mice (Walker et al., 1972; Thorpe and Walker, 1973; Tennekes et al., 1981; Tennekes et al., 1982), B6C3F1 mice (NCI, 1978a; Meierhenry et al., 1983), C57Bl/6J mice (Meierhenry et al., 1983), and C3H/HE mice (Meierhenry et al., 1983). These studies have identified a significantly increased incidence of liver tumors in exposed mice. An increase in liver tumors was identified in both males and females in all studies which evaluated both sexes. The NCI study was an exception and observed increases only in males. In one out of two studies in which other tissues in addition to the liver and lungs were stated to have been examined (Walker et al., 1972; NCI, 1978a), Walker et al. reported a significantly elevated incidence of lung tumors and lymphoid tumors in exposed females.

The lowest dose tested that increased the incidence of liver tumors was 1 ppm in females (Walker et al., 1972) and 2.5 ppm in males (Walker et al., 1972). The NCI also observed a significant increase in males at 5 ppm (NCI, 1978a). Walker et al. reported a significant increase of lung carcinoma and lymphoid tumors in female mice fed 0.1 ppm dieldrin and of lung adenoma in females fed 1.0 ppm. The increase in liver tumors was dose-related up to 10 ppm. The increase in the lung and lymphoid tumors was dose-related up to 1 ppm.

Earlier studies identified only benign hepatic tumors (Davis and Fitzhugh, 1962; Davis, 1965; MacDonald et al., 1972), however the tissue slides from these tests were re-evaluated by Reuber and other pathologists (Epstein, 1975) who identified malignant carcinoma in the hepatic cells. Later studies identified malignant, as well as benign liver tumors. Metastases were found in the lung tissue of some mice with malignant liver tumors by Walker et al. (1972), Thorpe and Walker (1973), NCI (1978a), and Meierhenry et al. (1983).

Tumor latency was also reduced by dieldrin exposure. Thorpe and Walker (1973) found liver tumors in a male fed 10 ppm dieldrin after 12 months of exposure, whereas in control mice, the earliest liver tumors were found in a male dying at 18 months and a female at 23 months. Tennekes et al. (1982), in a discussion of the Walker et al. and Thorpe and Walker data, reported that the median liver tumor induction period was significantly reduced by dieldrin at 0.1 ppm in female CF-1 mice and at 1.0 ppm in male CF-1 mice. The first liver carcinoma was observed in B6C3F1 male mice fed 5 ppm dieldrin at 88 weeks compared to 91 weeks in matched controls (NCI, 1978a). Meierhenry et al. (1983) observed liver tumors 10 weeks earlier in C57Bl/6J mice, 8 weeks earlier in B6C3F1 mice, and 25 weeks earlier in C3H/He mice compared to their respective control groups.

Rats

Studies have been conducted in four rat strains (Table 13), however only two bioassays in Osborne-Mendel and Fischer 344 rats were adequate to determine the carcinogenic potential of dieldrin exposure in rats (NCI 1978a and 1978b). These studies did not find an association of tumor incidence with dieldrin exposure using dietary dose levels between 2 and 65 ppm. On the other hand, the pathological results from one of the bioassays deemed inadequate for the evaluation of carcinogenicity (Fitzhugh et al., 1964) was reevaluated by Reuber (Epstein, 1975) who confirmed a statistically increased incidence of total multisite tumors in treated rats and diagnosed a statistically significant increase in liver cancer in males and females combined at 100 ppm in the diet. Reuber also reevaluated the pathological results in a subsample of animals studied by Deichmann et al. (1970) and found that the incidence of malignant tumors had been underestimated or under-reported by approximately three-fold. Finally, one of the bioassays conducted by the National Cancer

Institute (1978a) found a statistically significant (p = 0.007) increase in the combined incidence of adrenal cortical adenomas or carcinomas in low-dose females (6/45) versus pooled controls (0/55). The finding was discounted by the investigators because of a lack of dose-response and variability in other NCI studies. Therefore, although the results in two adequately conducted bioassays in rats have not associated dieldrin exposure with tumor causation, the body of experimental evidence has not been uniformly negative.

Hamsters

Male and female Syrian golden hamsters were fed 0 (40 males, 40 females), 20 (34 males, 33 females), 60 (32 males, 34 females), or 180 (41 males, 38 females) ppm dieldrin for their lifespans (Cabral et al., 1979). The last survivor was killed at 120 days of age. Autopsy was performed on all animals, and the thyroid, lungs, liver, kidney, spleen and gonads together with all organs showing macroscopical abnormalities were examined histologically.

Survival was low in all groups. At 70 weeks of age, only 30% of control males and 15% of control females were alive. Mortality was the lowest in the 180 ppm treated hamsters with 49% of males and 24% of females alive at 70 weeks. Males and females fed 20 and 180 ppm dieldrin were reported to show a marked retardation of growth, although the data were not presented. Although the percentage of tumor bearing animals was not significantly different between treated and control hamsters, more animals had more than one tumor in the treated than in the control groups. A hepatoma was found in one female and one male fed 180 ppm dieldrin, while no hepatomas were found in controls. Hepatic cell hypertrophy was observed in treated animals, and the incidence was dose-related. No conclusion regarding the carcinogenicity of dieldrin in hamsters can be drawn from this study because the high mortality rate in the animals may have precluded the development of tumors.

Dogs

The effects of long term administration of dieldrin to dogs for up to two years at doses ranging from 0.005 to 10 mg/kg/day were reported by three investigators. Treon and Cleveland (1955) fed diets containing 0, 1, or 3 ppm dieldrin to groups of two beagles per sex per dose. Fitzhugh et al. (1964) fed doses of 0.2 to 10 mg/kg/day six days a week to a total of 7 male and 7 female mongrel dogs. Walker et al. (1969) gave groups of five male and five female beagles daily oral does of 0.005 or 0.05 mg/kg/day dieldrin by capsule.

A dose-related increase in mortality occurred beginning at 0.5 mg/kg/day (Fitzhugh et al., 1964). No carcinogenic effects were reported by any of the investigators. The studies are not sufficient to determine carcinogenic effects because of the small sample sizes and the lack of a life-time exposure period.

Monkeys

Male rhesus monkeys (four years of age), five animals per group, were fed technical dieldrin (88.4% pure compound) in the diet at concentrations of 0, 0.01, 0.1, 0.5, 1.0 and 5.0 ppm (Wright et al., 1978). After four months, one of the monkeys in the 5 ppm group died of dieldrin intoxication. The dieldrin level in the highest dose group was then changed to 2.5 ppm. Another monkey in this dose group died of endocrine failure after six months of total exposure, and although the death was not considered to be related to dieldrin exposure, the dieldrin concentration in the diets of the three survivors was again reduced to 1.75 ppm at nine months. The dieldrin intake of one of the animals in this group was progressively increased until at 23 months of total exposure, his dietary concentration was 5 ppm. Two additional deaths, not related to dieldrin exposure, occurred over the course of the six year study. No dieldrin related effects, other than an increase in liver microsomal monooxygenase activity, was observed in the monkeys. No conclusions regarding the carcinogenic potential of dieldrin exposure in monkeys can be made on the basis of this study because of the small sample sizes used and the relative short duration of observation compared to the life-span of the monkey (20 years).

Table 12. Summary of Carcinogenesis Bioassays in Mice from U.S. EPA (1987).

TABLE 4-13. SUMMARY OF DIETARY CARCINOGENESIS BIOASSAYS OF DIELDRIN IN MICE

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
NS	C3HeB/Fe	215 M/F (treated) 217 M/F (controls)	0 (controls) 10	24 mo	Hepatomas: controls- 9/134 (7%) 10 ppm- 36/148 (24%)[a]	•Reevaluation by Reuber and other pathologists concluded that most liver tumors were hepatocellular carcinomas. •Limitations of study: -poor survival rate -many lost animals -data not tabulated by sex, time of death/tumors •Adequate test	Davis and Fitzhugh (1962) Reuber (as cited in Epstein, 1975)
NS	C3H	100 M&F/dose	0 10	24 mo	Hepatomas: controls-27/200 (13.5%) 10 ppm- 69/200 (34.5%)[b]	•Reevaluation by Reuber and other pathologists concluded that most liver tumors were hepatocellular carcinomas. •Limitations of study: -lack of detailed pathology -data not tabulated by sex, time of death/tumors •Adequate test	Davis (1965) Reuber (as cited in Epstein, 1975)
NS	C3HeB/FeJ CBA/J	55 treated (sex not specified); 10 controls (sex not specified)	0 15	375 days	Liver tumors: 7 treated mice had tumors	•Lack of detail as to sex, time of death, tumors, etc. •Limited value •Inadequate test	Song and Harville (1964)

(continued on the following page)

Table 12 continued.

TABLE 4-13. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
Technical Grade	Swiss-Webster	100 M/F/dose	0 3 10	Not clear	Hepatic Nodules: Dose-related 0 ppm-0% 3 ppm-2.5% 10 ppm-48%	•Reevaluation by Reuber and original pathologist. Many nodules reclassified as hepatocellular carcinomas. •Limitations of study: -only 71% histology examination -inadequate reporting of data •Adequate study	MacDonald et al. (1972) Reuber (as cited in Epstein, (1975)
>99% pure	CF-1	300 M/F (ctr) 125 M/F (0.1 ppm) 125 M/F (1.0 ppm) 200 M/F (10 ppm)	0 0.1 1 10	132 wk	Liver Tumors: Dose-related increased in type A, type B, and total liver tumors. Statistically significant ($p < 0.05$) at 10 ppm for both total liver tumors and type B tumors (carcinomas); lung, lymphoid and other tumors in female mice	•Carcinomas mainly in 10 ppm groups •Limitations of study -inadequate reporting of individual deaths and associated tumors •Adequate study	Walker et al. (1972)
>99% pure	CF-1	30 M/F/dose 78 M/F/controls	0 1.25 2.5 5 10 20	128 wk	Liver Tumors: Dose-related, except at 10-20 ppm where early mortality reduced number at risk	•Liver enlargements detected by 36 weeks. •20 ppm-high toxicity early deaths	Walker et al. (1972)

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TABLE 4-13. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
					(continued) 5 ppm Males- 87% (controls 12%) Females- 60% (controls 10%) Statistically significant ($p < 0.05$) at 2.5 ppm and above	(continued) •<10 ppm- minimal toxicity •No individual data •Adequate study	
>99% pure	CF-1	60 M/F/dose 78 M/F/controls	0 10	128 wk	Liver Tumors: Dose-related, increases in total tumors, type A and type B tumors	•Liver enlargements by 40-50 wk in all dose groups •Adequate study	Walker et al. (1972)
>99% pure	CF-1	29 M/F/dose	0 10	Varied from 2-64 wk	Liver Tumors: Significant increase ($p < 0.05$) with 64 wk of exposure; not significant for 32 or less wk of exposure	•Demonstrates need for long-term exposure to induce effect •Adequate study	Walker et al. (1972)
>99% pure	CF-1	30/M/F/dose 45 M/F/controls	0 10	110 wk	Liver Tumors: Significant increase ($p < 0.01$) in type B tumors of which many metastasized to the lungs Shortened latency period	•Liver enlargements by 50 wk; •Adequate study	Thorpe and Walker (1973)

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Table 12 continued.

TABLE 4-13. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
95% pure	B6C3F1	50 M&F/dose 20 M (controls) 10 F (controls)	0 2.5 5	80 wk treatment plus 10-13 wk observation	Hepatocellular Carcinomas: Males: HD-16/45 (36%)^a LD-12/49 (24%)^a Ctrl (M)-3/18(17%) Ctrl (P)-17/92(18%) Females: HD-3/46 (7%) LD-3/50 (6%) Ctrl (M)-1/18(6%) Ctrl (P)-7/91(8%)	•Dose-related mortality in females. •Adequate test	NCI (1978a)
>99% pure	CF-1	19-82 M/group	0 10	110 wk	Liver Tumors: 10 ppm-48/59 (81%) 0 ppm-6/60 (10%); 39% type B (carcinoma) at 10 ppm and only 1% in controls. Lung metastasia in 14% of 10 ppm animals; 0% for controls. Significant ($p < 0.01$)	•Limitations of study -Only males tested •Adequate study	Tennekes et al. (1981)
>99.9% pure	CF-1	1800 mice used in study. Number varied from 17 to 297 per groups equally divided by sex	0 0.1 1 2.5 5 10 20	Lifetime	Liver Tumors: Dose-related increase in incidences at 10 ppm and greater. Also dose-related reduction in induction time at 0.1 ppm and greater	•No linear relationship between median total dose or median time-to-tumor formation and daily exposure levels.	Tennekes et al. (1982)

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Table 12 continued.

TABLE 4-13. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
					(continued) In females and 1 in males.	(continued) •Authors suggest this indicates enhancement or promoting effect rather than initiation. •Adequate study	
>99% pure	CeH/He B6C3F1 C57/BL/6J	50/50 M 62/76 M 71/69 M (Dose/control groups)	0 10	85 wk exposure plus 47 wk observation (total=132 wk)	Hepatocellular Carcinomas: Significant increase ($p < 0.05$) in all 3 strains. •Also benign liver tumors in B6C3F1 and C57BL/6J mice	•Limitations of test -Only males tested •Adequate study	Meierhenry et al. (1983)

[a] Significantly different from controls ($p < 0.001$).

[b] Significantly different from controls ($p < 0.05$).

NS = Not specified; Ctrl (M) = matched controls; Ctrl (P) = pooled controls.

Table 13. Summary of Carcinogenesis Bioassays in Rats from U.S. EPA (1987).

TABLE 4-22. SUMMARY OF DIETARY CARCINOGENESIS BIOASSAYS OF DIELDRIN IN RATS

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
>85% pure	Carworth	40/sex/dose	0 2.5 12.5 25	2 yr	No treatment-related increase in tumors; No increased mortality or decreased growth in dieldrin-treated animals	•Limitations of study -Small numbers alive at 1 year; -No pathology data reported •Inadequate study	Treon and Cleveland (1955) Cleveland (1966)
100% pure	Osborne-Mendel	12/sex/group	0 0.5 2 10 50 100 150	2 yr	Dose-related decrease in mortality > 2 ppm. Dose-related increase in liver to body ratios; Chronic nephritis at high doses, esp. males; No liver tumors, although liver lesions > 50 ppm. Total tumors increased (24% in treated vs. 18% in controls)	•Reuber diagnosed liver cancers at 100 ppm and confirmed multiple-site tumors •Limitations of study -too few animals -high mortality > 2 ppm •Inadequate study	Fitzhugh et al. (1964) Reuber (as cited in Epstein (1975)
Unspecified purity	Holtzman	15/M or F/group	0 250 285	1 yr	No tumor data reported	•Limitations of study -too few animals -too short duration -lack of data reported •Inadequate study	Song and Harville (1964)

(continued on the following page)

Table 13 continued.

TABLE 4-22. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
>99% pure	Carworth	25/sex/treated group; 45/sex/treated/control	0 0.1 1 10	2 yr	No effect on body weight High mortality in all groups Dose-related increase in liver to body-weight ratio No treatment-related increase in tumors Liver pathology at 10 ppm	•Reevaluated by Stevenson-confirmed pathology results •Limitations of study -too few animals at risk > 1 year; -only 58% animals examined -individual animal data not presented •Inadequate study	Walker et al. (1969) Stevenson et al. (1976)
Technical Grade (purity not specified)	Osborne-Mendal	50/sex/dose 100/sex/controls	0 20 30 50	31 mo	No effect on body weight Survival decreased at 50 ppm Liver pathology; no increase in tumors with dieldrin exposure	•Reevaluation by Reuber on sample concluded Deichmann underestimated incidences of malignant tumors •Limitations of study -only 84% of animals microscopically examined -control histology not available •Minimally acceptable study	Deichmann et al. (1970) Reuber (as cited in Epstein, 1975)

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Table 13. continued.

TABLE 4-22. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
95% pure	Osborne-Mendel	50/sex/dose 10/sex matched controls 50 males and 60 females-colony controls	0 29 65	80 wk of exposure plus 30-31 wk of observation	Body weights lower in second year; Increase in mortality of treated rats; No treatment-related increase in tumors	•While increased mortality observed in treated groups, it was not significantly different at 18 months •No limitations of study •Adequate study	NCI (1978a)
Technical Grade (purified)	Fischer 344	24/sex	0 2 10 50	104-105 wk	No treatment-related change in body weight or mortality; No increase in tumors related to dieldrin exposure	•Limitations of study -Number of animals small •Adequate study	NCI (1978b)

IN VITRO EVIDENCE OF GENETIC AND EPIGENETIC MECHANISMS OF ACTION

The EPA Office of Pesticide Programs evaluated the published mutagenicity studies in June 1986 and the reports were included in the appendix to the U.S. EPA Carcinogenicity Assessment of Aldrin and Dieldrin (1987). Since more recent studies have not been published, the report and its conclusions are summarized in this section. More recent information regarding the effect of dieldrin exposure on cell to cell communication is available, and these studies are described as well.

Mutagenicity

The U.S. EPA (1987) classifies dieldrin as presumptively mutagenic in Salmonella. A single Ames test found an increase in the number of revertants in strains TA98 and TA100 in the presence and absence of mouse S9 activation. Positive results were also found in strain TA1535 in the presence of mouse S9 activation. Methodological problems with the study included a lack of positive controls and certain inconsistencies with other studies in background revertant frequency for the TA98 solvent controls and the TA1535 nonactivated solvent controls and the magnitude of the cytotoxic dose.

Three other Ames assays with sufficient primary data were found acceptable to conclude that nonactivated and rat or hamster S9-activated dieldrin is not mutagenic in *S. typhimurium*. Seven survey Ames tests also support this conclusion.

Nonactivated dieldrin was classified as an unconfirmed presumptive positive mutagen for Chinese hamster V79 cells, although there were reservations about the conclusion because of technical deficiencies. The deficiencies included; only one dose level of dieldrin, lack of replication, and lack of a positive control. In addition, carbaryl and 2,4-D, weak mutagens or nonmutagens in other tests, were reported to give strong positive responses.

Chromosome Aberrations

Dieldrin was classified as weakly clastogenic in mammalian somatic cells. Significant dose-related increases in the number of chromosomal aberrations (chromatid breaks, fragments, chromosome interchanges, and rings) were induced at all doses of dieldrin (nonactivated) in cultured human lung cells. A decrease in the mitotic index from 8.0 to 1.5% with increasing doses of dieldrin compared to control (11%) was also reported. The administration of single intraperitoneal doses of dieldrin to STS mice resulted in the finding of chromatid breaks, fragments, chromosome interchanges, and rings in harvested bone marrow cells. Decreases in the mitotic index from 6.0 to 2.8% with increasing doses of dieldrin compared to controls (10.5%) were also reported.

A similar study using HEOD and Chinese hamsters did not cause an increase in the number of chromosomal aberrations in bone marrow cells, however data concerning toxicity in the animals or cytotoxic effects were not given, and no positive control was used. The lymphocytes of dieldrin manufacturing workers were monitored by the same investigators and no differences in chromosomal aberrations were reported. The study population was very small - 17 presumable non-exposed, 9 former and 12 current plant workers - and no data concerning dieldrin exposure was provided.

HEOD, tested in a dominant lethal assay, did not cause a clastogenic response in male mouse germinal cells over a five week period. Another dominant lethal assay using dieldrin administered intraperitoneally or orally, did not result in any clastogenic effects in mouse germinal cells.

DNA Damage/Repair

Dieldrin did not cause unscheduled DNA synthesis in yeast or primary rat and mouse hepatocytes. Six independent assays of HEOD did not cause primary DNA damage in the bacterial species, Saccharomyces cerevisiae strain D4.

On the other hand, a statistically significant increase in UDS was induced by S9-activated and nonactivated dieldrin in SV-40 transformed human fibroblasts. The validity of the study was questioned because primary data for the two higher dose levels were not provided, thus precluding analysis of dose-related effects, and strong responses were induced by diquat, 2,4-D, and carbaryl, agents considered to be nongenotoxic or weakly genotoxic. Other technical difficulties further compromise the results. Dieldrin was classified as an inconclusive presumptive genotoxin in SV-40 transformed human cells.

Cell - Cell Communication

Wade et al. studied cell - cell communication indicated by gap fluorescence redistribution after photobleaching. Cultured human teratocarcinoma cells were labeled with 6-carboxy-fluorescein diacetate and exposed to a noncytotoxic dose of 7 ug/ml dieldrin. Pairs of contacting cells were selected and one of each pair was photobleached. The transfer of the dye from the unbleached cell to the bleached cell was measured over time using a series of image scans. Dieldrin blocked the fluorescence recover of photobleached cells. The fluorescence intensity of dieldrin-treated photobleached cells was approximately 1000 units fifteen minutes after bleaching compared to 14,000 units in control cells.

The same dose level of dieldrin (7 ug/ml) inhibited gap junction intercellular communication in rat glial cells after a 10 minute exposure of the cells analyzed by the FRAP method (Suter et al., 1987). The inhibition was shown to be reversible; cells treated for one hour with 7 ug/ml dieldrin and allowed to "recover" for four hours after the removal of the pesticide had established gap junction communication. The inhibition of gap junction communication was also tested in a scrape loading/dye transfer assay. A dose-related inhibition by dieldrin (0 - 10 ug/ml) was demonstrated. When a constant dose level, 7 ug/ml, dieldrin was administered to the cell for varying time periods, the extent of junctional communication was inversely correlated with the duration of treatment. Complete inhibition of dye transfer was observed after 50 minutes of treatment. The authors suggested that the inhibition of cell to cell communication may play a role in dieldrin induced neurotoxicity.

Dieldrin inhibited metabolic cooperation in 6-thioguanine resistant and sensitive human teratocarcinoma cell cultures (Zhong-Xiang et al., 1986). Wild-type human teratocarcinoma HTP3-4 cells (6-TG^s), 3X10^5 cells per plate, and 6-TG^r human teratocarcinoma HTXTG-1 cells, 200 cells per plate, were co-seeded. Five dishes per treatment group were used. Dieldrin was added to the plates after five hours and then 6-thioguanine was added two hours later. The cells were cultured in the presence of the pesticide for three days and in the 6-TG medium for an additional seven days. Dieldrin showed significant, dose-related inhibitory effects on metabolic cooperation at the noncytotoxic concentrations, 3 - 7 ug/ml. The recovery of 6-TG^r cells was 9% for negative controls, 27.5% for 3 ug/ml dieldrin, and reached a maximum of 88% for 7 ug/ml dieldrin. Dieldrin also inhibited the transfer of radioactive label between donor and recipient cells. Dieldrin was a more effective inhibitor than the positive control, TPA, in both assays. The authors postulated that altered calcium homeostasis may be involved in the inhibition of gap junction intercellular communication by the pesticide.

Trosko et al. (1987) evaluated the ability of dieldrin to inhibit gap junctional communication in an in vitro assay using co-cultures of Chinese hamster cells to measure metabolic cooperation between V79 6-thioguanine-sensitive ($6TG^s$) and resistant ($6TG^r$) cells. The cells were co-cultivated using 4×10^5 $6TG^s$ and 100 $6TG^r$ cell per plate. Five plates per dose of chemical were cultivated. Negative solvent control and positive control plates using TPA were also cultivated. Various concentrations of dieldrin (0 - 8 ug/ml) were added four hours after plating, and 6-thioguanine was added one hour later. Dieldrin was removed after three days. Nine days after cell plating, the colonies were counted. Dieldrin was not cytotoxic to the Chinese hamster V79 cells up to 7 ug/ml. Inhibition of metabolic cooperation increased in a dose-related manner between 5 and 8 ug/ml.

Klaunig and Ruch (1987) used a [^{3}H]uridine hepatocyte intercellular communication assay to study species and strain differences in response to dieldrin. Hepatocytes isolated from B6C3F1, C3H/HeN, C57Bl/6/N, and Balb/c/AnN strain mice, and F344 strain rats were evaluated. The percent of communicating recipient hepatocytes was determined by autoradiography eight hours after the cells were treated with dieldrin (1 ug/ml, 5 ug/ml, and 10 ug/ml in DMSO). Intercellular communication between donor and recipient cells was defined when the number of grains over donor-contacting recipients was at least twice that over each of three isolated recipients. The percent of communicating recipients was determined from at least 100 donor-contacting recipients for each culture. Triplicate cultures were examined for each treatment and strain of rodent.

Dieldrin significantly inhibited intercellular communication between B6C3Fl mouse, C3H mouse, and C57BL mouse hepatocytes at all dose levels and between Balb/c mouse hepatocytes at 5 and 10 ug/ml. Dieldrin did not affect intercellular between F344 rat hepatocytes at any of the concentrations examined. The B6C3Fl mouse hepatocytes were the most sensitive to the inhibitory effects of dieldrin. The authors noted that these results correlated with in vivo liver studies and concluded that tumor promoter induced inhibition of hepatocyte intercellular communication may be relevant to the in vivo hepatocarcinogenic process.

WEIGHT OF EVIDENCE CLASSIFICATION FOR DIELDRIN

Epidemiological Evidence

The epidemiologic data are inadequate to draw conclusions concerning the carcinogenicity of dieldrin in humans. Two retrospective cohort studies have failed to associate increased cancer incidence in insecticide manufacturing workers, however both had several limitations. The study populations were not characterized, the degree of dieldrin exposure within the study populations were not evaluated, sample sizes were small, and exposure to several chemicals was involved.

Studies in Experimental Animals

U.S.EPA's cancer guidelines state that an increased incidence of only mouse liver tumors will be regarded as sufficient evidence of carcinogenicity if all other conditions for a classification of "sufficient" evidence in animal studies are met. Factors that would lower the evidence classification to "limited" include lack of replication, increased incidence at high dose only or at the end of an experiment, no dose-related increase in malignancies, predominantly benign responses, no dose related shortening of the time to the appearance of tumors, negative or inconclusive results from a spectrum of short term tests for mutagenic activity, the occurrence of excess tumors only in a single sex.

The U.S. EPA's Science Advisory Board held a workshop on the relevance of mouse liver tumors and rat kidney tumors for predicting carcinogenicity in humans on August 12, 1987. The SAB concluded that, "for the most part, mouse liver tumors are indicative of human carcinogenicity" (Cothern, 1987). The supporting evidence, discussed at the workshop, is summarized below.

1. Mouse liver is the most common target site in the National Toxicology Program bioassays for a variety of chemicals. Although male mice have a high spontaneous rate of liver tumors, liver tumors in female mice is a more common target site. Female mice have a very low spontaneous incidence of liver tumors.
2. Mouse liver is one of the most common target sites in animals for compounds on the IARC list of potential human carcinogens, classified on the basis of epidemiologic studies and animal studies (not necessarily liver cancer in humans).
3. The pathology of mouse liver tumor resembles those in other species, including humans; and it goes through a sequence of histogenesis which is predictable, at least with spontaneous tumors, and those types of tumors such

as induced by DEN. A typical liver carcinoma in the mouse will metastasize to the lungs similarly to liver tumors in all other species including humans.

4. Mouse liver responds to well recognized carcinogens in a dose related fashion similarly to the response of other organs to carcinogens.
5. Multiplicity can be very important especially with increased incidence in one sex, in one strain of one species.
6. About 70% of chemicals that cause liver tumors in mice cause other kinds of tumors in mice or rats; and up to 70% of those mouse liver carcinogens are also mutagens.
7. The more organs that are involved and the more species that are involved, the more convincing is the evidence a chemical has carcinogenic potential in humans.

The positive studies of carcinogenicity in mice exposed to dieldrin are sufficient to place this persistent compound into the U.S. EPA weight of evidence classification of B2, a probable human carcinogen. Tumor incidence was significantly increased in seven different mice strains. Positive tumor incidence was attained in multiple tests for two strains, B6C3F1 mice and CF-1 mice. Although adequately conducted tests using rats did not result in evidence of carcinogenicity in this species, the nature of the neoplastic results do not allow downgrading the classification to "limited". Hepatocellular carcinomas as well as benign liver tumors were induced by dieldrin exposure, and these neoplasms had the ability to metastasize to the lungs. The cancer incidence was dose-related, and significant elevations in incidence occurred in lower dose groups. A significant decrease in the time to tumor appearance was also documented. Hepatocellular carcinoma was induced in both male and female mice.

A complete genetic toxicology profile has not been established for dieldrin (U.S. EPA, 1987). Although three acceptable Ames assays were negative for mutagenicity using rat or hamster S9-activated and nonactivated dieldrin, one test using mouse S9-activated dieldrin produced inconclusive results. Three *E. coli* reverse mutation survey studies were negative. An inconclusive mutagenic response was produced in Chinese hamster V79 cells. Dose related clastogenic responses were induced in both cultured human lung and mouse bone marrow cells. Dieldrin administered by gavage or intraperitoneally, was not clastogenic in male germinal cells. Primary DNA damage was not observed in bacteria, yeast or primary rat and mouse hepatocytes by dieldrin. However, S9-activated and nonactivated dieldrin induced unscheduled DNA synthesis in SV-40 transformed human fibroblasts. All studies showing positive evidence of activity were compromised by inadequate study designs or showed the greatest activity at cytotoxic doses. Several studies have demonstrated that dieldrin has the ability to interfere with cell gap junctional communication, a characteristic proposed for cancer promoters. Although short term assays suggest that dieldrin is not mutagenic, the inconclusive nature of a few studies, and the capability of dieldrin to inhibit cell-cell communication are evidence of dieldrin's cancer enhancing properties.

Pharmacodynamic studies in mice and rats indicate that there are differences between species, between strains, and between sexes. However, the excretion of dieldrin and metabolites in feces is the primary route of elimination in both mice and rats. The same metabolites are produced by the two species, however the relative proportions may differ. Although certain rat strains may metabolize and excrete dieldrin at a faster rate than mice, other rats strains have a slower rate of excretion. Finally, female rats metabolize and excrete dieldrin at a rate more similar to certain strains of mice than male rats of the same species. Therefore, it is not possible with the evidence that is available to ascribe the species difference in cancer causation to differences in the pharmacodynamic handling of dieldrin. Finally, humans appear to excrete dieldrin at a slower rate than either mice or rats suggesting greater susceptibility. There is no evidence to support the conclusion that either mice or rats are a more appropriate species for predicting the carcinogenic potential of dieldrin in humans.

DOSE RESPONSE ASSESSMENT

Calculation of the Reference Dose (RfD)

The liver has been identified as the most sensitive target organ for dieldrin toxicity. A lowest-adverse-effect-level (LOAEL) for liver toxicity in rats is 0.5 ppm, equivalent to 0.03 mg/kg/day assuming that rats consume 5% of their body weight. The LOAEL is based on elevated liver to body weight ratios and histological alterations in the hepatic cells of female Osborne-Mendel rats fed dieldrin for a lifetime with a diet containing 0.5 ppm dieldrin (Fitzhugh et al., 1967). A no-adverse-effect-level (NOAEL) for dieldrin has not been determined. A NOAEL or LOAEL for reproductive or developmental toxicity has not been determined. Dieldrin is a reproductive and developmental toxin at higher dose levels, but the compound has not been adequately tested for a variety of effects at dietary concentrations lower than 2.5 ppm.

Table 14 presents the Reference Dose (RfD) for noncarcinogenic toxicity, and the LOAEL, uncertainty factors (UF), and modifying factor (MF) upon which it is based. Two reference doses were calculated for each study. The RfD(total) represents the dose from all exposure sources expected to result in negligible effects. The RfD(fish) represents the dose from fish obtained by sport fishing expected to result in negligible effects in humans. This was determined by subtracting the dose of dieldrin from all other sources from the RfD(total). The dose from all other sources for dieldrin is 0.000018 mg/kg/day. This estimate is derived from the U.S. FDA market basket survey for 1981-82 (Gartrell et al., 1986) which measured residue levels in a typical adolescent male diet and the assumption that approximately 90% of the total dieldrin dose is derived from food.

Table 14. Parameters Used to Calculate the Reference Dose (RfD) for Systemic Effects in Humans Exposed to Dieldrin.

Reference	Target Organ	Dose mg/kg/day	End-point	Study Length months	UF[2]	MF[3]	Reference Dose mg/kg/day Total	Reference Dose mg/kg/day Fish
Fitzhugh et al., 1964	Liver	0.03	LOAEL[1]	24	10H 10A 10L	1	0.00003	0.000012

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[1] LOAEL = Lowest-Adverse-Effect-Level

[2] UF = Uncertainty factor. Uncertainty factors are used to calculate the reference dose. The no-adverse-effect-level (NOAEL) is divided by factors of 10 to account for variation in sensitivity among the members of the human population, 10H; uncertainty in extrapolating from animal data to humans, 10A; and uncertainty in extrapolating from a LOAEL instead of a NOAEL, 10L. See Table 5, Methods and Scientific Rationale for Hazard Assessment, Dose Response Assessment, and Risk Characterization for details.

[3] MF= Modifying factor. An additional uncertainty factor, greater than zero and less than or equal to 10, to further adjust for scientific uncertainties of the study.

Calculation of the Potency Factor (q^*_1) for Carcinogenicity

The U.S. EPA has found that thirteen data sets are suitable for a quantitative risk estimation. The data sets involve male and female C3H mice from Davis (1965) as reevaluated by Reuber, male and female CF-1 mice from Walker et al. (1972) and Thorpe and Walker (1973), male CF-1 mice from Tennekes et al.

(1981), male B6C3F1 mice from NCI (1978a and b), male C57Bl/fJ, C3H, and B6C3F1 mice from Meierhenry et al. (1983). Tables 15 through 27 are taken from the U.S. EPA Carcinogenicity Assessment of Aldrin and Dieldrin (1987) and present the data from each adequate study pertinent to the calculation of the human potency factor, q^*_1. The thirteen potency factors from the individual studies were averaged by calculating the geometric mean. An overall potency factor of 16 was determined.

The calculation of the geometric mean is supported by the conclusion that all six studies were adequate for risk estimation. There are reservations regarding this procedure which must be stated, however. Although all of these bioassays involved one species, mice, the study designs were not equivalent. Both the NCI (1978a) and Meierhenry et al. (1983) exposed the mice for less than the duration of the experiment. The resulting cancer incidence would be anticipated to be lower than incidence in bioassays involving a lifetime exposure. However, the exposure period in both studies was at least 80 weeks, the majority of the lifetime of the mouse. Therefore, the impact on the value of the potency factor was probably not significant.

The data used to calculate human potency did not account for the competing effect of treatment-related mortality even though survival was adversely affected in most of the bioassays. A method commonly used to partially account for differential mortality among dose groups is to present cancer incidence as a function of the number of animals that were alive at the time the first tumor was found in each group. These animals were at risk for tumor development whereas those dying prematurely were not. Ignoring the effects of mortality on cancer incidence would result in an underestimate of the potency factor. However, the cancer incidence in exposed male CF-1 mice was 100%

(Thorpe and Walker, 1973) and the potency factor calculated from this data serves as an upper limit for the estimates of human potency.

Finally, Meierhenry et al. (1983) killed animals (three per group) at intervals during the exposure period beginning only eight weeks after the initiation of the experiment. The time intervals and the number of animals killed prior to the end of the study were not stated. Therefore, it is possible that the number of animals at risk for cancer development was significantly less than the number of animals that began in each dose group, and tumor incidence was underestimated. Indeed, the potency factors estimated using this study were among the lowest of all the data sets. The geometric mean of potency factors from the adequate data sets excluding the three data sets from Meierhenry et al. (1983) is 20. The risk estimates using a potency factor of 20 are not different from those using a potency factor of 16 when numbers are rounded to one significant unit.

Therefore, despite the reservations discussed in this section, the potency factor, 16, derived by the U.S. EPA (1987) for mice, was used to estimate the upper 95% cancer risks for various consumption levels and fish species in the risk characterization section. Upper 95% cancer risks were also calculated using the potency factor, 55, derived from the response of the most sensitive sex and mouse strain.

Table 15. Data Sheet for Derivation of Potency Factor from U.S. EPA. (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mice, C3H, male
Body weight: 0.030 kg (assumed)
Length of experiment: 2 years
Length of exposure: 2 years
Tumor site and type: liver, carcinoma
Route, vehicle: oral, diet
Human potency: 22 per mg/kg/day

Experimental dose (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Incidence No. responding/ No. examined
0	0	0	22/73
10	1.3	0.104	62/71

SOURCE: Davis, 1965 (evaluated by Reuber; as cited in Epstein, 1975).

Table 16. Data Sheet for Derivation of Potency Factor from U.S. EPA (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

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npound: dieldrin
ecies, strain, sex: mice, C3H, female
dy weight: 0.030 kg (assumed)
igth of experiment: 2 years
igth of exposure: 2 years
nor site and type: liver, carcinoma
ite, vehicle: oral, diet
ian potency: 25 per mg/kg/day

erimental se (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Incidence No. responding/ No. examined
0	0	0	2/53
0	1.3	0.104	62/71

RCE: Davis, 1965 (evaluated by Reuber; as cited in Epstein, 1975).

Table 17. Data Sheet for Derivation of Potency Factor from U.S. EPA (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mice, CF-1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 33 months
Length of exposure: 24 months
Tumor site and type: liver, carcinoma
Route, vehicle: oral, diet
Human potency: 25 per mg/kg/day

Experimental dose (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Incidence No. responding/ No. examined
0	0	0	58/288
0.1	0.013	0.001	32/124
1	0.13	0.01	34/111
10	1.3	0.104	165/176

SOURCE: Walker et al., 1972.

Table 18. Data Sheet for Derivation of Potency Factor from U.S. EPA (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mice, CF-1, female
Body weight: 0.030 kg (assumed)
Length of experiment: 33 months
Length of exposure: 24 months
Tumor site and type: liver, carcinoma
Route, vehicle: oral, diet
Human potency: 28 per mg/kg/day

Experimental dose (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Incidence No. responding/ No. examined
0	0.0013	0.0001	39/297
0.1	0.013	0.001	24/90
1	0.13	0.01	32/87
10	1.3	0.104	136/148

SOURCE: Walker et al., 1972.

Table 19. Data Sheet for Derivation of Potency Factor from U.S. EPA (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, CF-1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 128 weeks
Length of exposure: 128 weeks
Tumor site and type: liver
Route, vehicle: oral, diet
Human potency: 15 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Tumor incidence
0	0	0	9/78
1.25	0.16	0.012	6/30
2.5	0.33	0.025	13/30
5	0.65	0.049	26/30
10	1.30	0.098	5/11
20	2.60	0.196	12/17

SOURCE: Walker et al., 1972.

Table 20. Data Sheet for Derivation of Potency Factor from U.S. EPA (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, CF-1, female
Body weight: 0.030 kg (assumed)
Length of experiment: 128 weeks
Length of exposure: 128 weeks
Tumor site and type: liver
Route, vehicle: oral, diet
Human potency: 7.1 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Tumor incidence
0	0	0	8/78
1.25	0.16	0.012	5/30
2.5	0.33	0.025	12/28
5	0.65	0.049	18/30
10	1.30	0.098	9/17
20	2.60	0.196	8/21

SOURCE: Walker et al., 1972.

Table 21. Data Sheet for Derivation of Potency Factor from U.S. EPA (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, CF-1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 110 weeks
Length of exposure: 110 weeks
Tumor site and type: liver
Route, vehicle: oral, diet
Human potency: 55 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)[a]	Tumor incidence[a]
0	0	0	11/45
10	1.30	0.098	30/30

[a] To calculate human potency, a tumor incidence of 29/30 was used and the dose was adjusted to 29/30 of the above value.

SOURCE: Thorpe and Walker, 1973.

Table 22. Data Sheet for Derivation of Potency Factors from U.S. EPA (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, CF-1, female
Body weight: 0.030 kg (assumed)
Length of experiment: 110 weeks
Length of exposure: 110 weeks
Tumor site and type: liver
Route, vehicle: oral, diet
Human potency: 26 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Tumor incidence
0	0	0	10/44
10	1.30	0.104	26/30

SOURCE: Thorpe and Walker, 1973.

Table 23. Data Sheet for Derivation of Potency Factor from U.S. EPA (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: technical-grade dieldrin
Species, strain, sex: mice, B6C3F1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 93 weeks
Length of exposure: 80 weeks
Tumor site and type: liver, carcinoma
Route, vehicle: oral, diet
Human potency: 9.8 per mg/kg/day

Experimental dose (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)[a]	Incidence No. responding/ No. examined
0	0	0	17/92
2.5	0.325	0.026	12/50
5	0.65	0.052	16/45

[a] Human equivalent doses were multiplied by 80/93 to reflect exposure less than the duration of the experiment.

SOURCE: NCI, 1978a.

Table 24. Data Sheet for Derivation of Potency Factor from U.S. EPA (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: Dieldrin
Species, strain, sex: mouse, CF-1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 110 weeks
Length of exposure: 110 weeks
Tumor site and type: liver
Route, vehicle: oral, diet
Human potency: 18 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Tumor incidence
0	0	0	25/252
10	1.30	0.104	113/139

SOURCE: Tennekes et al., 1981.

Table 25. Data Sheet for Derivation of Potency Factor from U.S. EPA (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, C57BL/6J, male
Body weight: 0.030 kg (assumed)
Length of experiment: 132 weeks
Length of exposure: 85 weeks
Tumor site and type: liver, hepatocellular carcinoma
Route, vehicle: oral, diet
Human potency: 7.4 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)[a]	Tumor incidence
0	0	0	0/69
10	1.30	0.104	21/71

[a] Human equivalent doses were multiplied by 85/132 to reflect exposure less than the duration of the experiment.

SOURCE: Meierhenry et al., 1983.

Table 26. Data Sheet for Derivation of Potency Factor from U.S. EPA (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, C3H/He, male
Body weight: 0.030 kg (assumed)
Length of experiment: 132 weeks
Length of exposure: 85 weeks
Tumor site and type: liver, hepatocellular carcinoma
Route, vehicle: oral, diet
Human potency: 8.5 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)[a]	Tumor incidence
0	0	0	6/50
10	1.30	0.104	19/50

[a] Human equivalent doses were multiplied by 85/132 to reflect exposure less than the duration of the experiment.

SOURCE: Meierhenry et al., 1983.

Table 27. Data Sheet for Derivation of Potencv Factor from U.S. EPA (1987).

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, B6C3F1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 132 weeks
Length of exposure: 85 weeks
Tumor site and type: liver, hepatocellular carcinoma
Route, vehicle: oral, diet
Human potency: 11 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)[a]	Tumor incidence
0	0	0	3/76
10	1.30	0.104	26/62

[a] Human equivalent doses were multiplied by 85/132 to reflect exposure less than the duration of the experiment.

SOURCE: Meierhenry et al., 1983.

RISK CHARACTERIZATION

Tables in the Appendix to this report present the upper 95% limit on cancer risk and hazard indices for each sport fish species using alternative values for meal size and meal frequency, and the average, minimum, and maximum tissue concentration in fish samples collected in 1985 and 1986. The tissue concentration data for both years were combined when they were available, or data from either year were used when a species was sampled in only one of those years (see exposure assessment section).

The estimates of cancer risk and other health hazards are for a 70 kg adult. It is important to note that children and lighter adults will have a higher dieldrin dose rate per meal and are more susceptible to health problems than these estimates indicate. For example, a 13.7 Kg (30 lb) toddler eating 12 quarter pound meals of brown trout (all sizes) per year, with have a dieldrin intake of 0.000026 mg/kg/day. This dose rate is five times higher than the dose for a 70 kg adult eating the same amount.

Hazard Indices and Contributions to Uncertainty

Table 29 presents the hazard index for each species for a meal size of 0.228 kg (0.5 lb) and a consumption frequency of one meal per month. At a consumption rate of one meal per month, the hazard indices based on mean tissue concentration are below one for 10 - 20 inch brown trout, 10 - 30 inch chinook, coho up to 30 inches, 10 - 20 inch walleye, and perch. Brown trout, 20 - 30 inches, and 10 - 20 inch whitefish have hazard indices of approximately one. The hazard indices of lake trout, greater than 10 inches, brown trout greater than 30 inches, and chinook salmon greater than 30 inches approach two and higher.

Tissue concentrations for dieldrin were nondetectable in individual fish from samples of several species. The range in tissue concentration results in a variation in hazard indices approximately one order of magnitude between the maximum and minimum tissue concentrations for each species. The variability was greater for 30 inch or longer chinook salmon for which the hazard index ranged from 0.09 to 9.1. The hazard indices for lake trout up to 30 inches ranged from 0 to about 5.

Upper 95% Limits on Cancer Risk Estimates and Contributions to Uncertainty

An upper 95% confidence limit for potency of 16 $(mg/kg/day)^{-1}$ was adopted from the geometric mean of the potency factors calculated from 13 data sets in male and female mice. This calculation was conducted by the U.S. EPA (1987). Some of the data sets included tumor incidence involving benign and malignant liver tumors combined (Walker et al., 1972; Thorpe and Walker, 1973; Tennekes et al., 1981). Potency factors based on the other studies involved hepatocellular carcinoma incidence only (Davis, 1965; NCI, 1978a; Meierhenry et al., 1983).

The potency factor based on the most sensitive sex and strain, male CF-1 mice, was 55 $(mg/kg/day)^{-1}$ (Thorpe and Walker, 1973). Benign tumors and hepatocellular carcinomas combined contribute to the estimate. One-hundred percent of the treated mice developed tumors, 53% of the mice had hepatocellular carcinomas. The potency factor based on this data set is 3.5 times greater than the potency factor based on the entire body of data available for mice.

A risk specific dose (RsD) derived from the maximum likelihood estimate (MLE) was compared to a risk specific dose based on the upper 95% confidence interval for two of the studies. The RsD based on the MLE for male mice in Walker et al. (1972) was two times greater than the RsD based on the 95% confidence limit. The RsD based on the MLE for male mice in NCI (1978a) was six times the RsD based on the upper 95% confidence interval. The values for the RsD based on the two estimation procedures were within an order of magnitude of each other.

Risk specific doses calculated using three different mathematical models (with an assumption of additive risk), multistage (Global 82), multihit, and weibull, were virtually identical using data for male mice in Walker et al. (1972). The data for male mice from NCI (1978a) resulted in a RsD calculated from the multistage model about three times higher than a RsD calculated from the multihit or weibull models.

The potency estimates were calculated using an assumed interspecies dose equivalency based on surface area. These estimates are about 14 times higher than potency factors calculated based on an assumed interspecies dose equivalency based on body weight.

The upper 95% limit on cancer risk resulting from the consumption of a 0.228 kg (0.5 lb) meal once a month or once a year are summarized for all sport fish species in Tables 30 and 31. The upper limit for cancer using average tissue concentration for a species (all size classes) ranges from about 3 x 10^{-5} for perch to 3 x 10^{-4} for lake trout when eaten once a month for a lifetime. The upper limit for cancer ranges from about 3 x 10^{-6} for perch to 2 x 10^{-5} for lake trout when eaten once a year for a lifetime. Fish species fall into the following rank order from lowest to highest risk; perch, walleye, coho salmon, chinook salmon, whitefish, brown trout, and lake trout.

Tissue concentration data for certain species showed a rising trend in tissue concentration with increasing length. Therefore, it was possible, for those species, to evaluate the change in risk for different size classes. The cancer risk from the consumption of lake trout longer than 30 inches is twice that of smaller lake trout. The upper bound cancer risk from the consumption of brown trout longer than 30 inches is 1.5 times that of 20 - 30 inch brown trout, which in turn, is twice that of 10 - 20 inch brown trout. The upper bound cancer risk from the consumption of chinook salmon longer than 30 inches is 5 times that of 20 - 30 inch chinook, which is 4 times that of 10 - 20 inch chinook.

The range in tissue concentration within a species causes the risk estimates to vary by up to two orders of magnitude (10 - 20 inch lake trout and chinook greater than 30 inches) for species and size classes in which detectable levels of dieldrin were obtained for all fish in the samples.

Tables 32, 33, and 34 present the number of meals per year that would result in an upper 95% cancer risk of 1 x 10^{-6}, 1 x 10^{-5}, and 1 x 10^{-4}. An upper limit risk of 1 x 10^{-6} is associated with the consumption of one 0.114 kg meal of chinook salmon less than 20 inches and less than one meal per week of other species. An upper limit risk of 1 x 10^{-5} is associated with the consumption of one to two 0.114 kg meals per year of brown trout or whitefish, 4 - 7 meals per year of coho salmon, walleye, or perch, and approximately one meal per month of chinook salmon less than 20 inches. Larger meals of these species could be eaten, but less frequently. An upper limit risk of 1 x 10^{-4} is associated with the consumption of one to many meals of individual species depending on the size of the meal eaten and the size class of the selected species.

Table 28.

Comparison of Hazard Indices (HI) for Various Lake Michigan Sport Fish Species Consumed Once a Month With Meal Size 0.228 Kg (0.5 lb). HI for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Hazard.

Species (Size Class)	HI Concentration Mean	Max.	Min.
Lake Trout (All Sizes)	1.52	5.44	0.00
Lake Trout (10 - 20 inches)	1.61	5.44	0.18
Lake Trout (20 - 30 inches)	1.61	4.91	0.00
Lake Trout (> 30 inches)	3.30	3.75	2.77
Brown Trout (All Sizes)	0.89	1.70	0.00
Brown Trout (10 - 20 inches)	0.54	1.07	0.00
Brown Trout (20 - 30 inches)	1.07	1.70	0.27
Brown Trout (> 30 inches)	1.61	1.61	1.61
Whitefish (10 - 20 inches)	0.80	1.16	0.45
Chinook (All Sizes)	0.62	9.10	0.00
Chinook (10 - 20 inches)	0.09	0.45	0.00
Chinook (20 - 30 inches)	0.36	0.62	0.00
Chinook (> 30 inches)	1.87	9.10	0.09
Coho (20 - 30 inches)	0.27	1.07	0.00
Walleye (10 - 20 inches)	0.18	0.71	0.00
Perch (All Sizes)	0.18	0.36	0.00

Table 29.

Comparison of the Upper 95% Limit on Cancer Risk for Various Lake Michigan Sport Fish Species Consumed Once a Month With Meal Size of 0.228 Kg (0.5 lb). Risk for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Risk.

	Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1		
Species (Size Class)	Mean	Max.	Min.
Lake Trout (All Sizes)	2.91E-04	1.05E-03	0.00E+00
Lake Trout (10 - 20 inches)	3.08E-04	1.05E-03	3.43E-05
Lake Trout (20 - 30 inches)	3.08E-04	9.42E-04	0.00E+00
Lake Trout (> 30 inches)	6.34E-04	7.20E-04	5.31E-04
Brown Trout (All Sizes)	1.71E-04	3.26E-04	0.00E+00
Brown Trout (10 - 20 inches)	1.03E-04	2.06E-04	0.00E+00
Brown Trout (20 - 30 inches)	2.06E-04	3.26E-04	5.14E-05
Brown Trout (> 30 inches)	3.08E-04	3.08E-04	3.08E-04
Whitefish (10 - 20 inches)	1.54E-04	2.23E-04	8.57E-05
Chinook (All Sizes)	1.20E-04	1.75E-03	0.00E+00
Chinook (10 - 20 inches)	1.71E-05	8.57E-05	0.00E+00
Chinook (20 - 30 inches)	6.85E-05	1.20E-04	0.00E+00
Chinook (> 30 inches)	3.60E-04	1.75E-03	1.71E-05
Coho (20 - 30 inches)	5.14E-05	2.06E-04	0.00E+00
Walleye (10 - 20 inches)	3.43E-05	1.37E-04	0.00E+00
Perch (All Sizes)	3.43E-05	6.85E-05	0.00E+00

Table 30.

Comparison of the Upper 95% Limit on Cancer Risk for Various Lake Michigan Sport Fish Species Consumed Once a Year With Meal Size of 0.228 Kg (0.5 lb). Risk for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Risk.

Species (Size Class)	Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1 Mean	Max.	Min.
Lake Trout (All Sizes)	2.43E-05	8.71E-05	0.00E+00
Lake Trout (10 - 20 inches)	2.57E-05	8.71E-05	2.86E-06
Lake Trout (20 - 30 inches)	2.57E-05	7.85E-05	0.00E+00
Lake Trout (> 30 inches)	5.28E-05	6.00E-05	4.43E-05
Brown Trout (All Sizes)	1.43E-05	2.71E-05	0.00E+00
Brown Trout (10 - 20 inches)	8.57E-06	1.71E-05	0.00E+00
Brown Trout (20 - 30 inches)	1.71E-05	2.71E-05	4.28E-06
Brown Trout (> 30 inches)	2.57E-05	2.57E-05	2.57E-05
Whitefish (10 - 20 inches)	1.29E-05	1.86E-05	7.14E-06
Chinook (All Sizes)	9.99E-06	1.46E-04	0.00E+00
Chinook (10 - 20 inches)	1.43E-06	7.14E-06	0.00E+00
Chinook (20 - 30 inches)	5.71E-06	9.99E-06	0.00E+00
Chinook (> 30 inches)	3.00E-05	1.46E-04	1.43E-06
Coho (All Sizes)	4.28E-06	1.71E-05	0.00E+00
Walleye (All Sizes)	2.86E-06	1.14E-05	0.00E+00
Perch (All Sizes)	2.86E-06	5.71E-06	0.00E+00

Table 31.

A Comparison of Meal Frequencies Resulting in an Upper 95% Limit on Cancer Risk of 1E-06, 1E-05, or 1E-04 For a Meal Size of 0.114 Kg of Various Lake Michigan Sport Fish Species Ranked by Decreasing Level of Risk.

Species (Size Class)	Meal Frequency (/Yr) Based on 1.00E-06 Risk	Meal Frequency (/Yr) Based on 1.00E-05 Risk	Meal Frequency (/Yr) Based on 1.00E-04 Risk
Lake Trout (All Sizes)	0.08	0.82	8.17
Lake Trout (10 - 20 inches)	0.08	0.77	7.72
Lake Trout (20 - 30 inches)	0.08	0.77	7.72
Lake Trout (> 30 inches)	0.04	0.38	3.76
Brown Trout (All Sizes)	0.14	1.39	13.90
Brown Trout (10 - 20 inches)	0.23	2.32	23.16
Brown Trout (20 - 30 inches)	0.12	1.16	11.58
Brown Trout (> 30 inches)	0.08	0.77	7.72
Whitefish (10 - 20 inches)	0.15	1.54	15.44
Chinook (All Sizes)	0.20	1.99	19.85
Chinook (10 - 20 inches)	1.39	13.90	138.96
Chinook (20 - 30 inches)	0.35	3.47	34.74
Chinook (> 30 inches)	0.07	0.66	6.62
Coho (20 - 30 inches)	0.46	4.63	46.32
Walleye (10 - 20 inches)	0.69	6.95	69.48
Perch (All Sizes)	0.69	6.95	69.48

Table 32.
A Comparison of Meal Frequencies Resulting in an Upper 95% Limit on Cancer Risk of 1E-06, 1E-05, or 1E-04 For a Meal Size of 0.228 Kg of Various Lake Michigan Sport Fish Species Ranked by Decreasing Level of Risk.

Species (Size Class)	Meal Frequency (/Yr)		
	Based on 1.00E-06 Risk	Based on 1.00E-05 Risk	Based on 1.00E-04 Risk
Lake Trout (All Sizes)	0.04	0.41	4.09
Lake Trout (10 - 20 inches)	0.04	0.39	3.86
Lake Trout (20 - 30 inches)	0.04	0.39	3.86
Lake Trout (> 30 inches)	0.02	0.19	1.88
			0.00
Brown Trout (All Sizes)	0.07	0.69	6.95
Brown Trout (10 - 20 inches)	0.12	1.16	11.58
Brown Trout (20 - 30 inches)	0.06	0.58	5.79
Brown Trout (> 30 inches)	0.04	0.39	3.86
			0.00
Whitefish (10 - 20 inches)	0.08	0.77	7.72
			0.00
Chinook (All Sizes)	0.10	0.99	9.93
Chinook (10 - 20 inches)	0.69	6.95	69.48
Chinook (20 - 30 inches)	0.17	1.74	17.37
Chinook (> 30 inches)	0.03	0.33	3.31
			0.00
Coho (20 - 30 inches)	0.23	2.32	23.16
			0.00
Walleye (10 - 20 inches)	0.35	3.47	34.74
			0.00
Perch (All Sizes)	0.35	3.47	34.74

Table 33.

A Comparison of Meal Frequencies Resulting in an Upper 95% Limit on Cancer Risk of 1E-06, 1E-05, or 1E-04 For a Meal Size of 0.34 Kg of Various Lake Michigan Sport Fish Species Ranked by Decreasing Level of Risk.

Species (Size Class)	Meal Frequency (/Yr) Based on 1.00E-06 Risk	Based on 1.00E-05 Risk	Based on 1.00E-04 Risk
Lake Trout (All Sizes)	0.03	0.27	2.74
Lake Trout (10 - 20 inches)	0.03	0.26	2.59
Lake Trout (20 - 30 inches)	0.03	0.26	2.59
Lake Trout (> 30 inches)	0.01	0.13	1.26
Brown Trout (All Sizes)	0.05	0.47	4.66
Brown Trout (10 - 20 inches)	0.08	0.78	7.77
Brown Trout (20 - 30 inches)	0.04	0.39	3.88
Brown Trout (> 30 inches)	0.03	0.26	2.59
Whitefish (10 - 20 inches)	0.05	0.52	5.18
Chinook (All Sizes)	0.07	0.67	6.66
Chinook (10 - 20 inches)	0.47	4.66	46.59
Chinook (20 - 30 inches)	0.12	1.16	11.65
Chinook (> 30 inches)	0.02	0.22	2.22
Coho (20 - 30 inches)	0.16	1.55	15.53
Walleye (10 - 20 inches)	0.23	2.33	23.30
Perch (All Sizes)	0.23	2.33	23.30

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CHAPTER 6 APPENDIX

Brown Trout (All Sizes)

Tissue Concentration (85 +86)	ppm
Mean	0.1
Max.	0.19
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000001	0.000000
0.114	2	0.000625	0.000001	0.000002	0.000000
0.114	4	0.001249	0.000002	0.000003	0.000000
0.114	6	0.001874	0.000003	0.000005	0.000000
0.114	12	0.003748	0.000005	0.000010	0.000000
0.114	24	0.007496	0.000011	0.000020	0.000000
0.114	52	0.016241	0.000023	0.000044	0.000000
0.114	104	0.032482	0.000046	0.000088	0.000000
0.114	156	0.048723	0.000070	0.000132	0.000000
0.228	1	0.000625	0.000001	0.000002	0.000000
0.228	2	0.001249	0.000002	0.000003	0.000000
0.228	4	0.002499	0.000004	0.000007	0.000000
0.228	6	0.003748	0.000005	0.000010	0.000000
0.228	12	0.007496	0.000011	0.000020	0.000000
0.228	24	0.014992	0.000021	0.000041	0.000000
0.228	52	0.032482	0.000046	0.000088	0.000000
0.228	104	0.064964	0.000093	0.000176	0.000000
0.228	156	0.097447	0.000139	0.000264	0.000000
0.34	1	0.000932	0.000001	0.000003	0.000000
0.34	2	0.001863	0.000003	0.000005	0.000000
0.34	4	0.003726	0.000005	0.000010	0.000000
0.34	6	0.005589	0.000008	0.000015	0.000000
0.34	12	0.011178	0.000016	0.000030	0.000000
0.34	24	0.022356	0.000032	0.000061	0.000000
0.34	52	0.048438	0.000069	0.000131	0.000000
0.34	104	0.096877	0.000138	0.000263	0.000000
0.34	156	0.145315	0.000208	0.000394	0.000000

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
7.14E-06	1.36E-05	0.00E+00	2.45E-05	4.66E-05	0.00E+00
1.43E-05	2.71E-05	0.00E+00	4.91E-05	9.33E-05	0.00E+00
2.86E-05	5.43E-05	0.00E+00	9.82E-05	1.87E-04	0.00E+00
4.28E-05	8.14E-05	0.00E+00	1.47E-04	2.80E-04	0.00E+00
8.57E-05	1.63E-04	0.00E+00	2.94E-04	5.60E-04	0.00E+00
1.71E-04	3.26E-04	0.00E+00	5.89E-04	1.12E-03	0.00E+00
3.71E-04	7.05E-04	0.00E+00	1.28E-03	2.42E-03	0.00E+00
7.42E-04	1.41E-03	0.00E+00	2.55E-03	4.85E-03	0.00E+00
1.11E-03	2.12E-03	0.00E+00	3.83E-03	7.27E-03	0.00E+00
1.43E-05	2.71E-05	0.00E+00	4.91E-05	9.33E-05	0.00E+00
2.86E-05	5.43E-05	0.00E+00	9.82E-05	1.87E-04	0.00E+00
5.71E-05	1.09E-04	0.00E+00	1.96E-04	3.73E-04	0.00E+00
8.57E-05	1.63E-04	0.00E+00	2.94E-04	5.60E-04	0.00E+00
1.71E-04	3.26E-04	0.00E+00	5.89E-04	1.12E-03	0.00E+00
3.43E-04	6.51E-04	0.00E+00	1.18E-03	2.24E-03	0.00E+00
7.42E-04	1.41E-03	0.00E+00	2.55E-03	4.85E-03	0.00E+00
1.48E-03	2.82E-03	0.00E+00	5.10E-03	9.70E-03	0.00E+00
2.23E-03	4.23E-03	0.00E+00	7.66E-03	1.45E-02	0.00E+00
2.13E-05	4.05E-05	0.00E+00	7.32E-05	1.39E-04	0.00E+00
4.26E-05	8.09E-05	0.00E+00	1.46E-04	2.78E-04	0.00E+00
8.52E-05	1.62E-04	0.00E+00	2.93E-04	5.56E-04	0.00E+00
1.28E-04	2.43E-04	0.00E+00	4.39E-04	8.34E-04	0.00E+00
2.55E-04	4.85E-04	0.00E+00	8.78E-04	1.67E-03	0.00E+00
5.11E-04	9.71E-04	0.00E+00	1.76E-03	3.34E-03	0.00E+00
1.11E-03	2.10E-03	0.00E+00	3.81E-03	7.23E-03	0.00E+00
2.21E-03	4.21E-03	0.00E+00	7.61E-03	1.45E-02	0.00E+00
3.32E-03	6.31E-03	0.00E+00	1.14E-02	2.17E-02	0.00E+00

```
====================================
            Hazard Index
      RfD =     0.000012 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
====================================

       0.04        0.07        0.00
       0.07        0.14        0.00
       0.15        0.28        0.00
       0.22        0.42        0.00
       0.45        0.85        0.00
       0.89        1.70        0.00
       1.93        3.67        0.00
       3.87        7.35        0.00
       5.80       11.02        0.00

       0.07        0.14        0.00
       0.15        0.28        0.00
       0.30        0.57        0.00
       0.45        0.85        0.00
       0.89        1.70        0.00
       1.78        3.39        0.00
       3.87        7.35        0.00
       7.73       14.69        0.00
      11.60       22.04        0.00

       0.11        0.21        0.00
       0.22        0.42        0.00
       0.44        0.84        0.00
       0.67        1.26        0.00
       1.33        2.53        0.00
       2.66        5.06        0.00
       5.77       10.96        0.00
      11.53       21.91        0.00
      17.30       32.87        0.00
```

Brown Trout (10 - 20 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.06
Max.	0.12
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000001	0.000000
0.114	2	0.000625	0.000001	0.000001	0.000000
0.114	4	0.001249	0.000001	0.000002	0.000000
0.114	6	0.001874	0.000002	0.000003	0.000000
0.114	12	0.003748	0.000003	0.000006	0.000000
0.114	24	0.007496	0.000006	0.000013	0.000000
0.114	52	0.016241	0.000014	0.000028	0.000000
0.114	104	0.032482	0.000028	0.000056	0.000000
0.114	156	0.048723	0.000042	0.000084	0.000000
0.228	1	0.000625	0.000001	0.000001	0.000000
0.228	2	0.001249	0.000001	0.000002	0.000000
0.228	4	0.002499	0.000002	0.000004	0.000000
0.228	6	0.003748	0.000003	0.000006	0.000000
0.228	12	0.007496	0.000006	0.000013	0.000000
0.228	24	0.014992	0.000013	0.000026	0.000000
0.228	52	0.032482	0.000028	0.000056	0.000000
0.228	104	0.064964	0.000056	0.000111	0.000000
0.228	156	0.097447	0.000084	0.000167	0.000000
0.34	1	0.000932	0.000001	0.000002	0.000000
0.34	2	0.001863	0.000002	0.000003	0.000000
0.34	4	0.003726	0.000003	0.000006	0.000000
0.34	6	0.005589	0.000005	0.000010	0.000000
0.34	12	0.011178	0.000010	0.000019	0.000000
0.34	24	0.022356	0.000019	0.000038	0.000000
0.34	52	0.048438	0.000042	0.000083	0.000000
0.34	104	0.096877	0.000083	0.000166	0.000000
0.34	156	0.145315	0.000125	0.000249	0.000000

Upper Limit (95%) on Risk			Upper Limit (95%) on Risk		
q* = 16 (Mg/Kg/Day)-1			q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
4.28E-06	8.57E-06	0.00E+00	1.47E-05	2.94E-05	0.00E+00
8.57E-06	1.71E-05	0.00E+00	2.94E-05	5.89E-05	0.00E+00
1.71E-05	3.43E-05	0.00E+00	5.89E-05	1.18E-04	0.00E+00
2.57E-05	5.14E-05	0.00E+00	8.83E-05	1.77E-04	0.00E+00
5.14E-05	1.03E-04	0.00E+00	1.77E-04	3.53E-04	0.00E+00
1.03E-04	2.06E-04	0.00E+00	3.53E-04	7.07E-04	0.00E+00
2.23E-04	4.45E-04	0.00E+00	7.66E-04	1.53E-03	0.00E+00
4.45E-04	8.91E-04	0.00E+00	1.53E-03	3.06E-03	0.00E+00
6.68E-04	1.34E-03	0.00E+00	2.30E-03	4.59E-03	0.00E+00
8.57E-06	1.71E-05	0.00E+00	2.94E-05	5.89E-05	0.00E+00
1.71E-05	3.43E-05	0.00E+00	5.89E-05	1.18E-04	0.00E+00
3.43E-05	6.85E-05	0.00E+00	1.18E-04	2.36E-04	0.00E+00
5.14E-05	1.03E-04	0.00E+00	1.77E-04	3.53E-04	0.00E+00
1.03E-04	2.06E-04	0.00E+00	3.53E-04	7.07E-04	0.00E+00
2.06E-04	4.11E-04	0.00E+00	7.07E-04	1.41E-03	0.00E+00
4.45E-04	8.91E-04	0.00E+00	1.53E-03	3.06E-03	0.00E+00
8.91E-04	1.78E-03	0.00E+00	3.06E-03	6.13E-03	0.00E+00
1.34E-03	2.67E-03	0.00E+00	4.59E-03	9.19E-03	0.00E+00
1.28E-05	2.55E-05	0.00E+00	4.39E-05	8.78E-05	0.00E+00
2.55E-05	5.11E-05	0.00E+00	8.78E-05	1.76E-04	0.00E+00
5.11E-05	1.02E-04	0.00E+00	1.76E-04	3.51E-04	0.00E+00
7.66E-05	1.53E-04	0.00E+00	2.63E-04	5.27E-04	0.00E+00
1.53E-04	3.07E-04	0.00E+00	5.27E-04	1.05E-03	0.00E+00
3.07E-04	6.13E-04	0.00E+00	1.05E-03	2.11E-03	0.00E+00
6.64E-04	1.33E-03	0.00E+00	2.28E-03	4.57E-03	0.00E+00
1.33E-03	2.66E-03	0.00E+00	4.57E-03	9.13E-03	0.00E+00
1.99E-03	3.99E-03	0.00E+00	6.85E-03	1.37E-02	0.00E+00

```
=======================================
            Hazard Index
      RfD =    0.000012 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
=======================================

       0.02        0.04        0.00
       0.04        0.09        0.00
       0.09        0.18        0.00
       0.13        0.27        0.00
       0.27        0.54        0.00
       0.54        1.07        0.00
       1.16        2.32        0.00
       2.32        4.64        0.00
       3.48        6.96        0.00

       0.04        0.09        0.00
       0.09        0.18        0.00
       0.18        0.36        0.00
       0.27        0.54        0.00
       0.54        1.07        0.00
       1.07        2.14        0.00
       2.32        4.64        0.00
       4.64        9.28        0.00
       6.96       13.92        0.00

       0.07        0.13        0.00
       0.13        0.27        0.00
       0.27        0.53        0.00
       0.40        0.80        0.00
       0.80        1.60        0.00
       1.60        3.19        0.00
       3.46        6.92        0.00
       6.92       13.84        0.00
      10.38       20.76        0.00
```

Brown Trout (20 - 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.12
Max.	0.19
Min.	0.03
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000001	0.000000
0.114	2	0.000625	0.000001	0.000002	0.000000
0.114	4	0.001249	0.000002	0.000003	0.000001
0.114	6	0.001874	0.000003	0.000005	0.000001
0.114	12	0.003748	0.000006	0.000010	0.000002
0.114	24	0.007496	0.000013	0.000020	0.000003
0.114	52	0.016241	0.000028	0.000044	0.000007
0.114	104	0.032482	0.000056	0.000088	0.000014
0.114	156	0.048723	0.000084	0.000132	0.000021
0.228	1	0.000625	0.000001	0.000002	0.000000
0.228	2	0.001249	0.000002	0.000003	0.000001
0.228	4	0.002499	0.000004	0.000007	0.000001
0.228	6	0.003748	0.000006	0.000010	0.000002
0.228	12	0.007496	0.000013	0.000020	0.000003
0.228	24	0.014992	0.000026	0.000041	0.000006
0.228	52	0.032482	0.000056	0.000088	0.000014
0.228	104	0.064964	0.000111	0.000176	0.000028
0.228	156	0.097447	0.000167	0.000264	0.000042
0.34	1	0.000932	0.000002	0.000003	0.000000
0.34	2	0.001863	0.000003	0.000005	0.000001
0.34	4	0.003726	0.000006	0.000010	0.000002
0.34	6	0.005589	0.000010	0.000015	0.000002
0.34	12	0.011178	0.000019	0.000030	0.000005
0.34	24	0.022356	0.000038	0.000061	0.000010
0.34	52	0.048438	0.000083	0.000131	0.000021
0.34	104	0.096877	0.000166	0.000263	0.000042
0.34	156	0.145315	0.000249	0.000394	0.000062

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
8.57E-06	1.36E-05	2.14E-06	2.94E-05	4.66E-05	7.36E-06
1.71E-05	2.71E-05	4.28E-06	5.89E-05	9.33E-05	1.47E-05
3.43E-05	5.43E-05	8.57E-06	1.18E-04	1.87E-04	2.94E-05
5.14E-05	8.14E-05	1.29E-05	1.77E-04	2.80E-04	4.42E-05
1.03E-04	1.63E-04	2.57E-05	3.53E-04	5.60E-04	8.83E-05
2.06E-04	3.26E-04	5.14E-05	7.07E-04	1.12E-03	1.77E-04
4.45E-04	7.05E-04	1.11E-04	1.53E-03	2.42E-03	3.83E-04
8.91E-04	1.41E-03	2.23E-04	3.06E-03	4.85E-03	7.66E-04
1.34E-03	2.12E-03	3.34E-04	4.59E-03	7.27E-03	1.15E-03
1.71E-05	2.71E-05	4.28E-06	5.89E-05	9.33E-05	1.47E-05
3.43E-05	5.43E-05	8.57E-06	1.18E-04	1.87E-04	2.94E-05
6.85E-05	1.09E-04	1.71E-05	2.36E-04	3.73E-04	5.89E-05
1.03E-04	1.63E-04	2.57E-05	3.53E-04	5.60E-04	8.83E-05
2.06E-04	3.26E-04	5.14E-05	7.07E-04	1.12E-03	1.77E-04
4.11E-04	6.51E-04	1.03E-04	1.41E-03	2.24E-03	3.53E-04
8.91E-04	1.41E-03	2.23E-04	3.06E-03	4.85E-03	7.66E-04
1.78E-03	2.82E-03	4.45E-04	6.13E-03	9.70E-03	1.53E-03
2.67E-03	4.23E-03	6.68E-04	9.19E-03	1.45E-02	2.30E-03
2.55E-05	4.05E-05	6.39E-06	8.78E-05	1.39E-04	2.20E-05
5.11E-05	8.09E-05	1.28E-05	1.76E-04	2.78E-04	4.39E-05
1.02E-04	1.62E-04	2.55E-05	3.51E-04	5.56E-04	8.78E-05
1.53E-04	2.43E-04	3.83E-05	5.27E-04	8.34E-04	1.32E-04
3.07E-04	4.85E-04	7.66E-05	1.05E-03	1.67E-03	2.63E-04
6.13E-04	9.71E-04	1.53E-04	2.11E-03	3.34E-03	5.27E-04
1.33E-03	2.10E-03	3.32E-04	4.57E-03	7.23E-03	1.14E-03
2.66E-03	4.21E-03	6.64E-04	9.13E-03	1.45E-02	2.28E-03
3.99E-03	6.31E-03	9.96E-04	1.37E-02	2.17E-02	3.43E-03

Hazard Index
RfD = 0.000012 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.04	0.07	0.01
0.09	0.14	0.02
0.18	0.28	0.04
0.27	0.42	0.07
0.54	0.85	0.13
1.07	1.70	0.27
2.32	3.67	0.58
4.64	7.35	1.16
6.96	11.02	1.74
0.09	0.14	0.02
0.18	0.28	0.04
0.36	0.57	0.09
0.54	0.85	0.13
1.07	1.70	0.27
2.14	3.39	0.54
4.64	7.35	1.16
9.28	14.69	2.32
13.92	22.04	3.48
0.13	0.21	0.03
0.27	0.42	0.07
0.53	0.84	0.13
0.80	1.26	0.20
1.60	2.53	0.40
3.19	5.06	0.80
6.92	10.96	1.73
13.84	21.91	3.46
20.76	32.87	5.19

Brown Trout (> 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.18
Max.	0.18
Min.	0.18
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000001	0.000001
0.114	2	0.000625	0.000002	0.000002	0.000002
0.114	4	0.001249	0.000003	0.000003	0.000003
0.114	6	0.001874	0.000005	0.000005	0.000005
0.114	12	0.003748	0.000010	0.000010	0.000010
0.114	24	0.007496	0.000019	0.000019	0.000019
0.114	52	0.016241	0.000042	0.000042	0.000042
0.114	104	0.032482	0.000084	0.000084	0.000084
0.114	156	0.048723	0.000125	0.000125	0.000125
0.228	1	0.000625	0.000002	0.000002	0.000002
0.228	2	0.001249	0.000003	0.000003	0.000003
0.228	4	0.002499	0.000006	0.000006	0.000006
0.228	6	0.003748	0.000010	0.000010	0.000010
0.228	12	0.007496	0.000019	0.000019	0.000019
0.228	24	0.014992	0.000039	0.000039	0.000039
0.228	52	0.032482	0.000084	0.000084	0.000084
0.228	104	0.064964	0.000167	0.000167	0.000167
0.228	156	0.097447	0.000251	0.000251	0.000251
0.34	1	0.000932	0.000002	0.000002	0.000002
0.34	2	0.001863	0.000005	0.000005	0.000005
0.34	4	0.003726	0.000010	0.000010	0.000010
0.34	6	0.005589	0.000014	0.000014	0.000014
0.34	12	0.011178	0.000029	0.000029	0.000029
0.34	24	0.022356	0.000057	0.000057	0.000057
0.34	52	0.048438	0.000125	0.000125	0.000125
0.34	104	0.096877	0.000249	0.000249	0.000249
0.34	156	0.145315	0.000374	0.000374	0.000374

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
1.29E-05	1.29E-05	1.29E-05	4.42E-05	4.42E-05	4.42E-05
2.57E-05	2.57E-05	2.57E-05	8.83E-05	8.83E-05	8.83E-05
5.14E-05	5.14E-05	5.14E-05	1.77E-04	1.77E-04	1.77E-04
7.71E-05	7.71E-05	7.71E-05	2.65E-04	2.65E-04	2.65E-04
1.54E-04	1.54E-04	1.54E-04	5.30E-04	5.30E-04	5.30E-04
3.08E-04	3.08E-04	3.08E-04	1.06E-03	1.06E-03	1.06E-03
6.68E-04	6.68E-04	6.68E-04	2.30E-03	2.30E-03	2.30E-03
1.34E-03	1.34E-03	1.34E-03	4.59E-03	4.59E-03	4.59E-03
2.00E-03	2.00E-03	2.00E-03	6.89E-03	6.89E-03	6.89E-03
2.57E-05	2.57E-05	2.57E-05	8.83E-05	8.83E-05	8.83E-05
5.14E-05	5.14E-05	5.14E-05	1.77E-04	1.77E-04	1.77E-04
1.03E-04	1.03E-04	1.03E-04	3.53E-04	3.53E-04	3.53E-04
1.54E-04	1.54E-04	1.54E-04	5.30E-04	5.30E-04	5.30E-04
3.08E-04	3.08E-04	3.08E-04	1.06E-03	1.06E-03	1.06E-03
6.17E-04	6.17E-04	6.17E-04	2.12E-03	2.12E-03	2.12E-03
1.34E-03	1.34E-03	1.34E-03	4.59E-03	4.59E-03	4.59E-03
2.67E-03	2.67E-03	2.67E-03	9.19E-03	9.19E-03	9.19E-03
4.01E-03	4.01E-03	4.01E-03	1.38E-02	1.38E-02	1.38E-02
3.83E-05	3.83E-05	3.83E-05	1.32E-04	1.32E-04	1.32E-04
7.66E-05	7.66E-05	7.66E-05	2.63E-04	2.63E-04	2.63E-04
1.53E-04	1.53E-04	1.53E-04	5.27E-04	5.27E-04	5.27E-04
2.30E-04	2.30E-04	2.30E-04	7.90E-04	7.90E-04	7.90E-04
4.60E-04	4.60E-04	4.60E-04	1.58E-03	1.58E-03	1.58E-03
9.20E-04	9.20E-04	9.20E-04	3.16E-03	3.16E-03	3.16E-03
1.99E-03	1.99E-03	1.99E-03	6.85E-03	6.85E-03	6.85E-03
3.99E-03	3.99E-03	3.99E-03	1.37E-02	1.37E-02	1.37E-02
5.98E-03	5.98E-03	5.98E-03	2.06E-02	2.06E-02	2.06E-02

Hazard Index
RfD = 0.000012 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.07	0.07	0.07
0.13	0.13	0.13
0.27	0.27	0.27
0.40	0.40	0.40
0.80	0.80	0.80
1.61	1.61	1.61
3.48	3.48	3.48
6.96	6.96	6.96
10.44	10.44	10.44
0.13	0.13	0.13
0.27	0.27	0.27
0.54	0.54	0.54
0.80	0.80	0.80
1.61	1.61	1.61
3.21	3.21	3.21
6.96	6.96	6.96
13.92	13.92	13.92
20.88	20.88	20.88
0.20	0.20	0.20
0.40	0.40	0.40
0.80	0.80	0.80
1.20	1.20	1.20
2.40	2.40	2.40
4.79	4.79	4.79
10.38	10.38	10.38
20.76	20.76	20.76
31.14	31.14	31.14

Chinook (10 - 20 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.01
Max.	0.05
Min.	0
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000000	0.000000
0.114	2	0.000625	0.000000	0.000000	0.000000
0.114	4	0.001249	0.000000	0.000001	0.000000
0.114	6	0.001874	0.000000	0.000001	0.000000
0.114	12	0.003748	0.000001	0.000003	0.000000
0.114	24	0.007496	0.000001	0.000005	0.000000
0.114	52	0.016241	0.000002	0.000012	0.000000
0.114	104	0.032482	0.000005	0.000023	0.000000
0.114	156	0.048723	0.000007	0.000035	0.000000
0.228	1	0.000625	0.000000	0.000000	0.000000
0.228	2	0.001249	0.000000	0.000001	0.000000
0.228	4	0.002499	0.000000	0.000002	0.000000
0.228	6	0.003748	0.000001	0.000003	0.000000
0.228	12	0.007496	0.000001	0.000005	0.000000
0.228	24	0.014992	0.000002	0.000011	0.000000
0.228	52	0.032482	0.000005	0.000023	0.000000
0.228	104	0.064964	0.000009	0.000046	0.000000
0.228	156	0.097447	0.000014	0.000070	0.000000
0.34	1	0.000932	0.000000	0.000001	0.000000
0.34	2	0.001863	0.000000	0.000001	0.000000
0.34	4	0.003726	0.000001	0.000003	0.000000
0.34	6	0.005589	0.000001	0.000004	0.000000
0.34	12	0.011178	0.000002	0.000008	0.000000
0.34	24	0.022356	0.000003	0.000016	0.000000
0.34	52	0.048438	0.000007	0.000035	0.000000
0.34	104	0.096877	0.000014	0.000069	0.000000
0.34	156	0.145315	0.000021	0.000104	0.000000

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
7.14E-07	3.57E-06	0.00E+00	2.45E-06	1.23E-05	0.00E+00
1.43E-06	7.14E-06	0.00E+00	4.91E-06	2.45E-05	0.00E+00
2.86E-06	1.43E-05	0.00E+00	9.82E-06	4.91E-05	0.00E+00
4.28E-06	2.14E-05	0.00E+00	1.47E-05	7.36E-05	0.00E+00
8.57E-06	4.28E-05	0.00E+00	2.94E-05	1.47E-04	0.00E+00
1.71E-05	8.57E-05	0.00E+00	5.89E-05	2.94E-04	0.00E+00
3.71E-05	1.86E-04	0.00E+00	1.28E-04	6.38E-04	0.00E+00
7.42E-05	3.71E-04	0.00E+00	2.55E-04	1.28E-03	0.00E+00
1.11E-04	5.57E-04	0.00E+00	3.83E-04	1.91E-03	0.00E+00
1.43E-06	7.14E-06	0.00E+00	4.91E-06	2.45E-05	0.00E+00
2.86E-06	1.43E-05	0.00E+00	9.82E-06	4.91E-05	0.00E+00
5.71E-06	2.86E-05	0.00E+00	1.96E-05	9.82E-05	0.00E+00
8.57E-06	4.28E-05	0.00E+00	2.94E-05	1.47E-04	0.00E+00
1.71E-05	8.57E-05	0.00E+00	5.89E-05	2.94E-04	0.00E+00
3.43E-05	1.71E-04	0.00E+00	1.18E-04	5.89E-04	0.00E+00
7.42E-05	3.71E-04	0.00E+00	2.55E-04	1.28E-03	0.00E+00
1.48E-04	7.42E-04	0.00E+00	5.10E-04	2.55E-03	0.00E+00
2.23E-04	1.11E-03	0.00E+00	7.66E-04	3.83E-03	0.00E+00
2.13E-06	1.06E-05	0.00E+00	7.32E-06	3.66E-05	0.00E+00
4.26E-06	2.13E-05	0.00E+00	1.46E-05	7.32E-05	0.00E+00
8.52E-06	4.26E-05	0.00E+00	2.93E-05	1.46E-04	0.00E+00
1.28E-05	6.39E-05	0.00E+00	4.39E-05	2.20E-04	0.00E+00
2.55E-05	1.28E-04	0.00E+00	8.78E-05	4.39E-04	0.00E+00
5.11E-05	2.55E-04	0.00E+00	1.76E-04	8.78E-04	0.00E+00
1.11E-04	5.54E-04	0.00E+00	3.81E-04	1.90E-03	0.00E+00
2.21E-04	1.11E-03	0.00E+00	7.61E-04	3.81E-03	0.00E+00
3.32E-04	1.66E-03	0.00E+00	1.14E-03	5.71E-03	0.00E+00

```
=====================================
            Hazard Index
      RfD =    0.000012 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
=====================================

       0.00        0.02        0.00
       0.01        0.04        0.00
       0.01        0.07        0.00
       0.02        0.11        0.00
       0.04        0.22        0.00
       0.09        0.45        0.00
       0.19        0.97        0.00
       0.39        1.93        0.00
       0.58        2.90        0.00

       0.01        0.04        0.00
       0.01        0.07        0.00
       0.03        0.15        0.00
       0.04        0.22        0.00
       0.09        0.45        0.00
       0.18        0.89        0.00
       0.39        1.93        0.00
       0.77        3.87        0.00
       1.16        5.80        0.00

       0.01        0.06        0.00
       0.02        0.11        0.00
       0.04        0.22        0.00
       0.07        0.33        0.00
       0.13        0.67        0.00
       0.27        1.33        0.00
       0.58        2.88        0.00
       1.15        5.77        0.00
       1.73        8.65        0.00
```

Chinook (20 - 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.04
Max.	0.07
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000000	0.000000
0.114	2	0.000625	0.000000	0.000001	0.000000
0.114	4	0.001249	0.000001	0.000001	0.000000
0.114	6	0.001874	0.000001	0.000002	0.000000
0.114	12	0.003748	0.000002	0.000004	0.000000
0.114	24	0.007496	0.000004	0.000007	0.000000
0.114	52	0.016241	0.000009	0.000016	0.000000
0.114	104	0.032482	0.000019	0.000032	0.000000
0.114	156	0.048723	0.000028	0.000049	0.000000
0.228	1	0.000625	0.000000	0.000001	0.000000
0.228	2	0.001249	0.000001	0.000001	0.000000
0.228	4	0.002499	0.000001	0.000002	0.000000
0.228	6	0.003748	0.000002	0.000004	0.000000
0.228	12	0.007496	0.000004	0.000007	0.000000
0.228	24	0.014992	0.000009	0.000015	0.000000
0.228	52	0.032482	0.000019	0.000032	0.000000
0.228	104	0.064964	0.000037	0.000065	0.000000
0.228	156	0.097447	0.000056	0.000097	0.000000
0.34	1	0.000932	0.000001	0.000001	0.000000
0.34	2	0.001863	0.000001	0.000002	0.000000
0.34	4	0.003726	0.000002	0.000004	0.000000
0.34	6	0.005589	0.000003	0.000006	0.000000
0.34	12	0.011178	0.000006	0.000011	0.000000
0.34	24	0.022356	0.000013	0.000022	0.000000
0.34	52	0.048438	0.000028	0.000048	0.000000
0.34	104	0.096877	0.000055	0.000097	0.000000
0.34	156	0.145315	0.000083	0.000145	0.000000

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
2.86E-06	5.00E-06	0.00E+00	9.82E-06	1.72E-05	0.00E+00
5.71E-06	9.99E-06	0.00E+00	1.96E-05	3.44E-05	0.00E+00
1.14E-05	2.00E-05	0.00E+00	3.93E-05	6.87E-05	0.00E+00
1.71E-05	3.00E-05	0.00E+00	5.89E-05	1.03E-04	0.00E+00
3.43E-05	6.00E-05	0.00E+00	1.18E-04	2.06E-04	0.00E+00
6.85E-05	1.20E-04	0.00E+00	2.36E-04	4.12E-04	0.00E+00
1.48E-04	2.60E-04	0.00E+00	5.10E-04	8.93E-04	0.00E+00
2.97E-04	5.20E-04	0.00E+00	1.02E-03	1.79E-03	0.00E+00
4.45E-04	7.80E-04	0.00E+00	1.53E-03	2.68E-03	0.00E+00
5.71E-06	9.99E-06	0.00E+00	1.96E-05	3.44E-05	0.00E+00
1.14E-05	2.00E-05	0.00E+00	3.93E-05	6.87E-05	0.00E+00
2.28E-05	4.00E-05	0.00E+00	7.85E-05	1.37E-04	0.00E+00
3.43E-05	6.00E-05	0.00E+00	1.18E-04	2.06E-04	0.00E+00
6.85E-05	1.20E-04	0.00E+00	2.36E-04	4.12E-04	0.00E+00
1.37E-04	2.40E-04	0.00E+00	4.71E-04	8.25E-04	0.00E+00
2.97E-04	5.20E-04	0.00E+00	1.02E-03	1.79E-03	0.00E+00
5.94E-04	1.04E-03	0.00E+00	2.04E-03	3.57E-03	0.00E+00
8.91E-04	1.56E-03	0.00E+00	3.06E-03	5.36E-03	0.00E+00
8.52E-06	1.49E-05	0.00E+00	2.93E-05	5.12E-05	0.00E+00
1.70E-05	2.98E-05	0.00E+00	5.86E-05	1.02E-04	0.00E+00
3.41E-05	5.96E-05	0.00E+00	1.17E-04	2.05E-04	0.00E+00
5.11E-05	8.94E-05	0.00E+00	1.76E-04	3.07E-04	0.00E+00
1.02E-04	1.79E-04	0.00E+00	3.51E-04	6.15E-04	0.00E+00
2.04E-04	3.58E-04	0.00E+00	7.03E-04	1.23E-03	0.00E+00
4.43E-04	7.75E-04	0.00E+00	1.52E-03	2.66E-03	0.00E+00
8.86E-04	1.55E-03	0.00E+00	3.04E-03	5.33E-03	0.00E+00
1.33E-03	2.33E-03	0.00E+00	4.57E-03	7.99E-03	0.00E+00

```
====================================
          Hazard Index
     RfD =     0.000012 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
====================================

       0.01        0.03        0.00
       0.03        0.05        0.00
       0.06        0.10        0.00
       0.09        0.16        0.00
       0.18        0.31        0.00
       0.36        0.62        0.00
       0.77        1.35        0.00
       1.55        2.71        0.00
       2.32        4.06        0.00

       0.03        0.05        0.00
       0.06        0.10        0.00
       0.12        0.21        0.00
       0.18        0.31        0.00
       0.36        0.62        0.00
       0.71        1.25        0.00
       1.55        2.71        0.00
       3.09        5.41        0.00
       4.64        8.12        0.00

       0.04        0.08        0.00
       0.09        0.16        0.00
       0.18        0.31        0.00
       0.27        0.47        0.00
       0.53        0.93        0.00
       1.06        1.86        0.00
       2.31        4.04        0.00
       4.61        8.07        0.00
       6.92       12.11        0.00
```

Chinook (> 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.21
Max.	1.02
Min.	0.01
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000005	0.000000
0.114	2	0.000625	0.000002	0.000009	0.000000
0.114	4	0.001249	0.000004	0.000018	0.000000
0.114	6	0.001874	0.000006	0.000027	0.000000
0.114	12	0.003748	0.000011	0.000055	0.000001
0.114	24	0.007496	0.000022	0.000109	0.000001
0.114	52	0.016241	0.000049	0.000237	0.000002
0.114	104	0.032482	0.000097	0.000473	0.000005
0.114	156	0.048723	0.000146	0.000710	0.000007
0.228	1	0.000625	0.000002	0.000009	0.000000
0.228	2	0.001249	0.000004	0.000018	0.000000
0.228	4	0.002499	0.000007	0.000036	0.000000
0.228	6	0.003748	0.000011	0.000055	0.000001
0.228	12	0.007496	0.000022	0.000109	0.000001
0.228	24	0.014992	0.000045	0.000218	0.000002
0.228	52	0.032482	0.000097	0.000473	0.000005
0.228	104	0.064964	0.000195	0.000947	0.000009
0.228	156	0.097447	0.000292	0.001420	0.000014
0.34	1	0.000932	0.000003	0.000014	0.000000
0.34	2	0.001863	0.000006	0.000027	0.000000
0.34	4	0.003726	0.000011	0.000054	0.000001
0.34	6	0.005589	0.000017	0.000081	0.000001
0.34	12	0.011178	0.000034	0.000163	0.000002
0.34	24	0.022356	0.000067	0.000326	0.000003
0.34	52	0.048438	0.000145	0.000706	0.000007
0.34	104	0.096877	0.000291	0.001412	0.000014
0.34	156	0.145315	0.000436	0.002117	0.000021

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
1.50E-05	7.28E-05	7.14E-07	5.15E-05	2.50E-04	2.45E-06
3.00E-05	1.46E-04	1.43E-06	1.03E-04	5.01E-04	4.91E-06
6.00E-05	2.91E-04	2.86E-06	2.06E-04	1.00E-03	9.82E-06
9.00E-05	4.37E-04	4.28E-06	3.09E-04	1.50E-03	1.47E-05
1.80E-04	8.74E-04	8.57E-06	6.18E-04	3.00E-03	2.94E-05
3.60E-04	1.75E-03	1.71E-05	1.24E-03	6.01E-03	5.89E-05
7.80E-04	3.79E-03	3.71E-05	2.68E-03	1.30E-02	1.28E-04
1.56E-03	7.57E-03	7.42E-05	5.36E-03	2.60E-02	2.55E-04
2.34E-03	1.14E-02	1.11E-04	8.04E-03	3.90E-02	3.83E-04
3.00E-05	1.46E-04	1.43E-06	1.03E-04	5.01E-04	4.91E-06
6.00E-05	2.91E-04	2.86E-06	2.06E-04	1.00E-03	9.82E-06
1.20E-04	5.83E-04	5.71E-06	4.12E-04	2.00E-03	1.96E-05
1.80E-04	8.74E-04	8.57E-06	6.18E-04	3.00E-03	2.94E-05
3.60E-04	1.75E-03	1.71E-05	1.24E-03	6.01E-03	5.89E-05
7.20E-04	3.50E-03	3.43E-05	2.47E-03	1.20E-02	1.18E-04
1.56E-03	7.57E-03	7.42E-05	5.36E-03	2.60E-02	2.55E-04
3.12E-03	1.51E-02	1.48E-04	1.07E-02	5.21E-02	5.10E-04
4.68E-03	2.27E-02	2.23E-04	1.61E-02	7.81E-02	7.66E-04
4.47E-05	2.17E-04	2.13E-06	1.54E-04	7.47E-04	7.32E-06
8.94E-05	4.34E-04	4.26E-06	3.07E-04	1.49E-03	1.46E-05
1.79E-04	8.69E-04	8.52E-06	6.15E-04	2.99E-03	2.93E-05
2.68E-04	1.30E-03	1.28E-05	9.22E-04	4.48E-03	4.39E-05
5.37E-04	2.61E-03	2.55E-05	1.84E-03	8.96E-03	8.78E-05
1.07E-03	5.21E-03	5.11E-05	3.69E-03	1.79E-02	1.76E-04
2.33E-03	1.13E-02	1.11E-04	7.99E-03	3.88E-02	3.81E-04
4.65E-03	2.26E-02	2.21E-04	1.60E-02	7.76E-02	7.61E-04
6.98E-03	3.39E-02	3.32E-04	2.40E-02	1.16E-01	1.14E-03

```
========================================
           Hazard Index
      RfD =     0.000012 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
========================================

       0.08        0.38        0.00
       0.16        0.76        0.01
       0.31        1.52        0.01
       0.47        2.28        0.02
       0.94        4.55        0.04
       1.87        9.10        0.09
       4.06       19.72        0.19
       8.12       39.44        0.39
      12.18       59.16        0.58

       0.16        0.76        0.01
       0.31        1.52        0.01
       0.62        3.03        0.03
       0.94        4.55        0.04
       1.87        9.10        0.09
       3.75       18.20        0.18
       8.12       39.44        0.39
      16.24       78.89        0.77
      24.36      118.33        1.16

       0.23        1.13        0.01
       0.47        2.26        0.02
       0.93        4.52        0.04
       1.40        6.79        0.07
       2.79       13.57        0.13
       5.59       27.15        0.27
      12.11       58.82        0.58
      24.22      117.64        1.15
      36.33      176.45        1.73
```

Chinook (All Sizes)

Tissue Concentration (85 +86)	ppm
Mean	0.07
Max.	1.02
Min.	0
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000005	0.000000
0.114	2	0.000625	0.000001	0.000009	0.000000
0.114	4	0.001249	0.000001	0.000018	0.000000
0.114	6	0.001874	0.000002	0.000027	0.000000
0.114	12	0.003748	0.000004	0.000055	0.000000
0.114	24	0.007496	0.000007	0.000109	0.000000
0.114	52	0.016241	0.000016	0.000237	0.000000
0.114	104	0.032482	0.000032	0.000473	0.000000
0.114	156	0.048723	0.000049	0.000710	0.000000
0.228	1	0.000625	0.000001	0.000009	0.000000
0.228	2	0.001249	0.000001	0.000018	0.000000
0.228	4	0.002499	0.000002	0.000036	0.000000
0.228	6	0.003748	0.000004	0.000055	0.000000
0.228	12	0.007496	0.000007	0.000109	0.000000
0.228	24	0.014992	0.000015	0.000218	0.000000
0.228	52	0.032482	0.000032	0.000473	0.000000
0.228	104	0.064964	0.000065	0.000947	0.000000
0.228	156	0.097447	0.000097	0.001420	0.000000
0.34	1	0.000932	0.000001	0.000014	0.000000
0.34	2	0.001863	0.000002	0.000027	0.000000
0.34	4	0.003726	0.000004	0.000054	0.000000
0.34	6	0.005589	0.000006	0.000081	0.000000
0.34	12	0.011178	0.000011	0.000163	0.000000
0.34	24	0.022356	0.000022	0.000326	0.000000
0.34	52	0.048438	0.000048	0.000706	0.000000
0.34	104	0.096877	0.000097	0.001412	0.000000
0.34	156	0.145315	0.000145	0.002117	0.000000

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
5.00E-06	7.28E-05	0.00E+00	1.72E-05	2.50E-04	0.00E+00
9.99E-06	1.46E-04	0.00E+00	3.44E-05	5.01E-04	0.00E+00
2.00E-05	2.91E-04	0.00E+00	6.87E-05	1.00E-03	0.00E+00
3.00E-05	4.37E-04	0.00E+00	1.03E-04	1.50E-03	0.00E+00
6.00E-05	8.74E-04	0.00E+00	2.06E-04	3.00E-03	0.00E+00
1.20E-04	1.75E-03	0.00E+00	4.12E-04	6.01E-03	0.00E+00
2.60E-04	3.79E-03	0.00E+00	8.93E-04	1.30E-02	0.00E+00
5.20E-04	7.57E-03	0.00E+00	1.79E-03	2.60E-02	0.00E+00
7.80E-04	1.14E-02	0.00E+00	2.68E-03	3.90E-02	0.00E+00
9.99E-06	1.46E-04	0.00E+00	3.44E-05	5.01E-04	0.00E+00
2.00E-05	2.91E-04	0.00E+00	6.87E-05	1.00E-03	0.00E+00
4.00E-05	5.83E-04	0.00E+00	1.37E-04	2.00E-03	0.00E+00
6.00E-05	8.74E-04	0.00E+00	2.06E-04	3.00E-03	0.00E+00
1.20E-04	1.75E-03	0.00E+00	4.12E-04	6.01E-03	0.00E+00
2.40E-04	3.50E-03	0.00E+00	8.25E-04	1.20E-02	0.00E+00
5.20E-04	7.57E-03	0.00E+00	1.79E-03	2.60E-02	0.00E+00
1.04E-03	1.51E-02	0.00E+00	3.57E-03	5.21E-02	0.00E+00
1.56E-03	2.27E-02	0.00E+00	5.36E-03	7.81E-02	0.00E+00
1.49E-05	2.17E-04	0.00E+00	5.12E-05	7.47E-04	0.00E+00
2.98E-05	4.34E-04	0.00E+00	1.02E-04	1.49E-03	0.00E+00
5.96E-05	8.69E-04	0.00E+00	2.05E-04	2.99E-03	0.00E+00
8.94E-05	1.30E-03	0.00E+00	3.07E-04	4.48E-03	0.00E+00
1.79E-04	2.61E-03	0.00E+00	6.15E-04	8.96E-03	0.00E+00
3.58E-04	5.21E-03	0.00E+00	1.23E-03	1.79E-02	0.00E+00
7.75E-04	1.13E-02	0.00E+00	2.66E-03	3.88E-02	0.00E+00
1.55E-03	2.26E-02	0.00E+00	5.33E-03	7.76E-02	0.00E+00
2.33E-03	3.39E-02	0.00E+00	7.99E-03	1.16E-01	0.00E+00

```
====================================
            Hazard Index
      RfD =    0.000012 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
====================================

       0.03        0.38        0.00
       0.05        0.76        0.00
       0.10        1.52        0.00
       0.16        2.28        0.00
       0.31        4.55        0.00
       0.62        9.10        0.00
       1.35       19.72        0.00
       2.71       39.44        0.00
       4.06       59.16        0.00

       0.05        0.76        0.00
       0.10        1.52        0.00
       0.21        3.03        0.00
       0.31        4.55        0.00
       0.62        9.10        0.00
       1.25       18.20        0.00
       2.71       39.44        0.00
       5.41       78.89        0.00
       8.12      118.33        0.00

       0.08        1.13        0.00
       0.16        2.26        0.00
       0.31        4.52        0.00
       0.47        6.79        0.00
       0.93       13.57        0.00
       1.86       27.15        0.00
       4.04       58.82        0.00
       8.07      117.64        0.00
      12.11      176.45        0.00
```

Coho (20 - 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.03
Max.	0.12
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000001	0.000000
0.114	2	0.000625	0.000000	0.000001	0.000000
0.114	4	0.001249	0.000001	0.000002	0.000000
0.114	6	0.001874	0.000001	0.000003	0.000000
0.114	12	0.003748	0.000002	0.000006	0.000000
0.114	24	0.007496	0.000003	0.000013	0.000000
0.114	52	0.016241	0.000007	0.000028	0.000000
0.114	104	0.032482	0.000014	0.000056	0.000000
0.114	156	0.048723	0.000021	0.000084	0.000000
0.228	1	0.000625	0.000000	0.000001	0.000000
0.228	2	0.001249	0.000001	0.000002	0.000000
0.228	4	0.002499	0.000001	0.000004	0.000000
0.228	6	0.003748	0.000002	0.000006	0.000000
0.228	12	0.007496	0.000003	0.000013	0.000000
0.228	24	0.014992	0.000006	0.000026	0.000000
0.228	52	0.032482	0.000014	0.000056	0.000000
0.228	104	0.064964	0.000028	0.000111	0.000000
0.228	156	0.097447	0.000042	0.000167	0.000000
0.34	1	0.000932	0.000000	0.000002	0.000000
0.34	2	0.001863	0.000001	0.000003	0.000000
0.34	4	0.003726	0.000002	0.000006	0.000000
0.34	6	0.005589	0.000002	0.000010	0.000000
0.34	12	0.011178	0.000005	0.000019	0.000000
0.34	24	0.022356	0.000010	0.000038	0.000000
0.34	52	0.048438	0.000021	0.000083	0.000000
0.34	104	0.096877	0.000042	0.000166	0.000000
0.34	156	0.145315	0.000062	0.000249	0.000000

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
2.14E-06	8.57E-06	0.00E+00	7.36E-06	2.94E-05	0.00E+00
4.28E-06	1.71E-05	0.00E+00	1.47E-05	5.89E-05	0.00E+00
8.57E-06	3.43E-05	0.00E+00	2.94E-05	1.18E-04	0.00E+00
1.29E-05	5.14E-05	0.00E+00	4.42E-05	1.77E-04	0.00E+00
2.57E-05	1.03E-04	0.00E+00	8.83E-05	3.53E-04	0.00E+00
5.14E-05	2.06E-04	0.00E+00	1.77E-04	7.07E-04	0.00E+00
1.11E-04	4.45E-04	0.00E+00	3.83E-04	1.53E-03	0.00E+00
2.23E-04	8.91E-04	0.00E+00	7.66E-04	3.06E-03	0.00E+00
3.34E-04	1.34E-03	0.00E+00	1.15E-03	4.59E-03	0.00E+00
4.28E-06	1.71E-05	0.00E+00	1.47E-05	5.89E-05	0.00E+00
8.57E-06	3.43E-05	0.00E+00	2.94E-05	1.18E-04	0.00E+00
1.71E-05	6.85E-05	0.00E+00	5.89E-05	2.36E-04	0.00E+00
2.57E-05	1.03E-04	0.00E+00	8.83E-05	3.53E-04	0.00E+00
5.14E-05	2.06E-04	0.00E+00	1.77E-04	7.07E-04	0.00E+00
1.03E-04	4.11E-04	0.00E+00	3.53E-04	1.41E-03	0.00E+00
2.23E-04	8.91E-04	0.00E+00	7.66E-04	3.06E-03	0.00E+00
4.45E-04	1.78E-03	0.00E+00	1.53E-03	6.13E-03	0.00E+00
6.68E-04	2.67E-03	0.00E+00	2.30E-03	9.19E-03	0.00E+00
6.39E-06	2.55E-05	0.00E+00	2.20E-05	8.78E-05	0.00E+00
1.28E-05	5.11E-05	0.00E+00	4.39E-05	1.76E-04	0.00E+00
2.55E-05	1.02E-04	0.00E+00	8.78E-05	3.51E-04	0.00E+00
3.83E-05	1.53E-04	0.00E+00	1.32E-04	5.27E-04	0.00E+00
7.66E-05	3.07E-04	0.00E+00	2.63E-04	1.05E-03	0.00E+00
1.53E-04	6.13E-04	0.00E+00	5.27E-04	2.11E-03	0.00E+00
3.32E-04	1.33E-03	0.00E+00	1.14E-03	4.57E-03	0.00E+00
6.64E-04	2.66E-03	0.00E+00	2.28E-03	9.13E-03	0.00E+00
9.96E-04	3.99E-03	0.00E+00	3.43E-03	1.37E-02	0.00E+00

```
========================================
             Hazard Index
       RfD =    0.000012 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
========================================

         0.01        0.04        0.00
         0.02        0.09        0.00
         0.04        0.18        0.00
         0.07        0.27        0.00
         0.13        0.54        0.00
         0.27        1.07        0.00
         0.58        2.32        0.00
         1.16        4.64        0.00
         1.74        6.96        0.00

         0.02        0.09        0.00
         0.04        0.18        0.00
         0.09        0.36        0.00
         0.13        0.54        0.00
         0.27        1.07        0.00
         0.54        2.14        0.00
         1.16        4.64        0.00
         2.32        9.28        0.00
         3.48       13.92        0.00

         0.03        0.13        0.00
         0.07        0.27        0.00
         0.13        0.53        0.00
         0.20        0.80        0.00
         0.40        1.60        0.00
         0.80        3.19        0.00
         1.73        6.92        0.00
         3.46       13.84        0.00
         5.19       20.76        0.00
```

Lake Trout (10 - 20 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.18
Max.	0.61
Min.	0.02
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000003	0.000000
0.114	2	0.000625	0.000002	0.000005	0.000000
0.114	4	0.001249	0.000003	0.000011	0.000000
0.114	6	0.001874	0.000005	0.000016	0.000001
0.114	12	0.003748	0.000010	0.000033	0.000001
0.114	24	0.007496	0.000019	0.000065	0.000002
0.114	52	0.016241	0.000042	0.000142	0.000005
0.114	104	0.032482	0.000084	0.000283	0.000009
0.114	156	0.048723	0.000125	0.000425	0.000014
0.228	1	0.000625	0.000002	0.000005	0.000000
0.228	2	0.001249	0.000003	0.000011	0.000000
0.228	4	0.002499	0.000006	0.000022	0.000001
0.228	6	0.003748	0.000010	0.000033	0.000001
0.228	12	0.007496	0.000019	0.000065	0.000002
0.228	24	0.014992	0.000039	0.000131	0.000004
0.228	52	0.032482	0.000084	0.000283	0.000009
0.228	104	0.064964	0.000167	0.000566	0.000019
0.228	156	0.097447	0.000251	0.000849	0.000028
0.34	1	0.000932	0.000002	0.000008	0.000000
0.34	2	0.001863	0.000005	0.000016	0.000001
0.34	4	0.003726	0.000010	0.000032	0.000001
0.34	6	0.005589	0.000014	0.000049	0.000002
0.34	12	0.011178	0.000029	0.000097	0.000003
0.34	24	0.022356	0.000057	0.000195	0.000006
0.34	52	0.048438	0.000125	0.000422	0.000014
0.34	104	0.096877	0.000249	0.000844	0.000028
0.34	156	0.145315	0.000374	0.001266	0.000042

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
1.29E-05	4.35E-05	1.43E-06	4.42E-05	1.50E-04	4.91E-06
2.57E-05	8.71E-05	2.86E-06	8.83E-05	2.99E-04	9.82E-06
5.14E-05	1.74E-04	5.71E-06	1.77E-04	5.99E-04	1.96E-05
7.71E-05	2.61E-04	8.57E-06	2.65E-04	8.98E-04	2.94E-05
1.54E-04	5.23E-04	1.71E-05	5.30E-04	1.80E-03	5.89E-05
3.08E-04	1.05E-03	3.43E-05	1.06E-03	3.59E-03	1.18E-04
6.68E-04	2.26E-03	7.42E-05	2.30E-03	7.78E-03	2.55E-04
1.34E-03	4.53E-03	1.48E-04	4.59E-03	1.56E-02	5.10E-04
2.00E-03	6.79E-03	2.23E-04	6.89E-03	2.34E-02	7.66E-04
2.57E-05	8.71E-05	2.86E-06	8.83E-05	2.99E-04	9.82E-06
5.14E-05	1.74E-04	5.71E-06	1.77E-04	5.99E-04	1.96E-05
1.03E-04	3.48E-04	1.14E-05	3.53E-04	1.20E-03	3.93E-05
1.54E-04	5.23E-04	1.71E-05	5.30E-04	1.80E-03	5.89E-05
3.08E-04	1.05E-03	3.43E-05	1.06E-03	3.59E-03	1.18E-04
6.17E-04	2.09E-03	6.85E-05	2.12E-03	7.19E-03	2.36E-04
1.34E-03	4.53E-03	1.48E-04	4.59E-03	1.56E-02	5.10E-04
2.67E-03	9.06E-03	2.97E-04	9.19E-03	3.11E-02	1.02E-03
4.01E-03	1.36E-02	4.45E-04	1.38E-02	4.67E-02	1.53E-03
3.83E-05	1.30E-04	4.26E-06	1.32E-04	4.46E-04	1.46E-05
7.66E-05	2.60E-04	8.52E-06	2.63E-04	8.93E-04	2.93E-05
1.53E-04	5.20E-04	1.70E-05	5.27E-04	1.79E-03	5.86E-05
2.30E-04	7.79E-04	2.55E-05	7.90E-04	2.68E-03	8.78E-05
4.60E-04	1.56E-03	5.11E-05	1.58E-03	5.36E-03	1.76E-04
9.20E-04	3.12E-03	1.02E-04	3.16E-03	1.07E-02	3.51E-04
1.99E-03	6.75E-03	2.21E-04	6.85E-03	2.32E-02	7.61E-04
3.99E-03	1.35E-02	4.43E-04	1.37E-02	4.64E-02	1.52E-03
5.98E-03	2.03E-02	6.64E-04	2.06E-02	6.96E-02	2.28E-03

Hazard Index

RfD = 0.000012 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.07	0.23	0.00
0.13	0.45	0.00
0.27	0.91	0.00
0.40	1.36	0.00
0.80	2.72	0.00
1.61	5.44	0.00
3.48	11.79	0.00
6.96	23.59	0.00
10.44	35.38	0.00
0.13	0.45	0.00
0.27	0.91	0.00
0.54	1.81	0.00
0.80	2.72	0.00
1.61	5.44	0.00
3.21	10.89	0.00
6.96	23.59	0.00
13.92	47.18	0.00
20.88	70.76	0.00
0.20	0.68	0.01
0.40	1.35	0.03
0.80	2.71	0.04
1.20	4.06	0.09
2.40	8.12	0.18
4.79	16.23	0.39
10.38	35.18	0.77
20.76	70.35	1.16
31.14	105.53	0.00

Lake Trout (20 - 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.18
Max.	0.55
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000002	0.000000
0.114	2	0.000625	0.000002	0.000005	0.000000
0.114	4	0.001249	0.000003	0.000010	0.000000
0.114	6	0.001874	0.000005	0.000015	0.000000
0.114	12	0.003748	0.000010	0.000029	0.000000
0.114	24	0.007496	0.000019	0.000059	0.000000
0.114	52	0.016241	0.000042	0.000128	0.000000
0.114	104	0.032482	0.000084	0.000255	0.000000
0.114	156	0.048723	0.000125	0.000383	0.000000
0.228	1	0.000625	0.000002	0.000005	0.000000
0.228	2	0.001249	0.000003	0.000010	0.000000
0.228	4	0.002499	0.000006	0.000020	0.000000
0.228	6	0.003748	0.000010	0.000029	0.000000
0.228	12	0.007496	0.000019	0.000059	0.000000
0.228	24	0.014992	0.000039	0.000118	0.000000
0.228	52	0.032482	0.000084	0.000255	0.000000
0.228	104	0.064964	0.000167	0.000510	0.000000
0.228	156	0.097447	0.000251	0.000766	0.000000
0.34	1	0.000932	0.000002	0.000007	0.000000
0.34	2	0.001863	0.000005	0.000015	0.000000
0.34	4	0.003726	0.000010	0.000029	0.000000
0.34	6	0.005589	0.000014	0.000044	0.000000
0.34	12	0.011178	0.000029	0.000088	0.000000
0.34	24	0.022356	0.000057	0.000176	0.000000
0.34	52	0.048438	0.000125	0.000381	0.000000
0.34	104	0.096877	0.000249	0.000761	0.000000
0.34	156	0.145315	0.000374	0.001142	0.000000

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==================================================================================
      Upper Limit (95%) on Risk                 Upper Limit (95%) on Risk
         q* = 16 (Mg/Kg/Day)-1                     q* = 55 (Mg/Kg/Day)-1
       Mean          Max.          Min.          Mean          Max.          Min.
==================================================================================


   1.29E-05      3.93E-05      0.00E+00      4.42E-05      1.35E-04      0.00E+00
   2.57E-05      7.85E-05      0.00E+00      8.83E-05      2.70E-04      0.00E+00
   5.14E-05      1.57E-04      0.00E+00      1.77E-04      5.40E-04      0.00E+00
   7.71E-05      2.36E-04      0.00E+00      2.65E-04      8.10E-04      0.00E+00
   1.54E-04      4.71E-04      0.00E+00      5.30E-04      1.62E-03      0.00E+00
   3.08E-04      9.42E-04      0.00E+00      1.06E-03      3.24E-03      0.00E+00
   6.68E-04      2.04E-03      0.00E+00      2.30E-03      7.02E-03      0.00E+00
   1.34E-03      4.08E-03      0.00E+00      4.59E-03      1.40E-02      0.00E+00
   2.00E-03      6.13E-03      0.00E+00      6.89E-03      2.11E-02      0.00E+00

   2.57E-05      7.85E-05      0.00E+00      8.83E-05      2.70E-04      0.00E+00
   5.14E-05      1.57E-04      0.00E+00      1.77E-04      5.40E-04      0.00E+00
   1.03E-04      3.14E-04      0.00E+00      3.53E-04      1.08E-03      0.00E+00
   1.54E-04      4.71E-04      0.00E+00      5.30E-04      1.62E-03      0.00E+00
   3.08E-04      9.42E-04      0.00E+00      1.06E-03      3.24E-03      0.00E+00
   6.17E-04      1.88E-03      0.00E+00      2.12E-03      6.48E-03      0.00E+00
   1.34E-03      4.08E-03      0.00E+00      4.59E-03      1.40E-02      0.00E+00
   2.67E-03      8.17E-03      0.00E+00      9.19E-03      2.81E-02      0.00E+00
   4.01E-03      1.23E-02      0.00E+00      1.38E-02      4.21E-02      0.00E+00

   3.83E-05      1.17E-04      0.00E+00      1.32E-04      4.03E-04      0.00E+00
   7.66E-05      2.34E-04      0.00E+00      2.63E-04      8.05E-04      0.00E+00
   1.53E-04      4.68E-04      0.00E+00      5.27E-04      1.61E-03      0.00E+00
   2.30E-04      7.03E-04      0.00E+00      7.90E-04      2.42E-03      0.00E+00
   4.60E-04      1.41E-03      0.00E+00      1.58E-03      4.83E-03      0.00E+00
   9.20E-04      2.81E-03      0.00E+00      3.16E-03      9.66E-03      0.00E+00
   1.99E-03      6.09E-03      0.00E+00      6.85E-03      2.09E-02      0.00E+00
   3.99E-03      1.22E-02      0.00E+00      1.37E-02      4.19E-02      0.00E+00
   5.98E-03      1.83E-02      0.00E+00      2.06E-02      6.28E-02      0.00E+00
```

Hazard Index		
RfD =	0.000012 Liver	
Mean Conc.	Max. Conc.	Min. Conc.
0.07	0.20	0.00
0.13	0.41	0.00
0.27	0.82	0.00
0.40	1.23	0.00
0.80	2.45	0.00
1.61	4.91	0.00
3.48	10.63	0.00
6.96	21.27	0.00
10.44	31.90	0.00
0.13	0.41	0.00
0.27	0.82	0.00
0.54	1.64	0.00
0.80	2.45	0.00
1.61	4.91	0.00
3.21	9.82	0.00
6.96	21.27	0.00
13.92	42.54	0.00
20.88	63.80	0.00
0.20	0.61	0.00
0.40	1.22	0.00
0.80	2.44	0.00
1.20	3.66	0.00
2.40	7.32	0.00
4.79	14.64	0.00
10.38	31.72	0.00
20.76	63.43	0.00
31.14	95.15	0.00

Lake Trout (> 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.37
Max.	0.42
Min.	0.31
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000002	0.000002	0.000001
0.114	2	0.000625	0.000003	0.000004	0.000003
0.114	4	0.001249	0.000007	0.000007	0.000006
0.114	6	0.001874	0.000010	0.000011	0.000008
0.114	12	0.003748	0.000020	0.000022	0.000017
0.114	24	0.007496	0.000040	0.000045	0.000033
0.114	52	0.016241	0.000086	0.000097	0.000072
0.114	104	0.032482	0.000172	0.000195	0.000144
0.114	156	0.048723	0.000258	0.000292	0.000216
0.228	1	0.000625	0.000003	0.000004	0.000003
0.228	2	0.001249	0.000007	0.000007	0.000006
0.228	4	0.002499	0.000013	0.000015	0.000011
0.228	6	0.003748	0.000020	0.000022	0.000017
0.228	12	0.007496	0.000040	0.000045	0.000033
0.228	24	0.014992	0.000079	0.000090	0.000066
0.228	52	0.032482	0.000172	0.000195	0.000144
0.228	104	0.064964	0.000343	0.000390	0.000288
0.228	156	0.097447	0.000515	0.000585	0.000432
0.34	1	0.000932	0.000005	0.000006	0.000004
0.34	2	0.001863	0.000010	0.000011	0.000008
0.34	4	0.003726	0.000020	0.000022	0.000017
0.34	6	0.005589	0.000030	0.000034	0.000025
0.34	12	0.011178	0.000059	0.000067	0.000050
0.34	24	0.022356	0.000118	0.000134	0.000099
0.34	52	0.048438	0.000256	0.000291	0.000215
0.34	104	0.096877	0.000512	0.000581	0.000429
0.34	156	0.145315	0.000768	0.000872	0.000644

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
2.64E-05	3.00E-05	2.21E-05	9.08E-05	1.03E-04	7.61E-05
5.28E-05	6.00E-05	4.43E-05	1.82E-04	2.06E-04	1.52E-04
1.06E-04	1.20E-04	8.85E-05	3.63E-04	4.12E-04	3.04E-04
1.58E-04	1.80E-04	1.33E-04	5.45E-04	6.18E-04	4.56E-04
3.17E-04	3.60E-04	2.66E-04	1.09E-03	1.24E-03	9.13E-04
6.34E-04	7.20E-04	5.31E-04	2.18E-03	2.47E-03	1.83E-03
1.37E-03	1.56E-03	1.15E-03	4.72E-03	5.36E-03	3.96E-03
2.75E-03	3.12E-03	2.30E-03	9.44E-03	1.07E-02	7.91E-03
4.12E-03	4.68E-03	3.45E-03	1.42E-02	1.61E-02	1.19E-02
5.28E-05	6.00E-05	4.43E-05	1.82E-04	2.06E-04	1.52E-04
1.06E-04	1.20E-04	8.85E-05	3.63E-04	4.12E-04	3.04E-04
2.11E-04	2.40E-04	1.77E-04	7.26E-04	8.25E-04	6.09E-04
3.17E-04	3.60E-04	2.66E-04	1.09E-03	1.24E-03	9.13E-04
6.34E-04	7.20E-04	5.31E-04	2.18E-03	2.47E-03	1.83E-03
1.27E-03	1.44E-03	1.06E-03	4.36E-03	4.95E-03	3.65E-03
2.75E-03	3.12E-03	2.30E-03	9.44E-03	1.07E-02	7.91E-03
5.49E-03	6.24E-03	4.60E-03	1.89E-02	2.14E-02	1.58E-02
8.24E-03	9.35E-03	6.90E-03	2.83E-02	3.22E-02	2.37E-02
7.88E-05	8.94E-05	6.60E-05	2.71E-04	3.07E-04	2.27E-04
1.58E-04	1.79E-04	1.32E-04	5.42E-04	6.15E-04	4.54E-04
3.15E-04	3.58E-04	2.64E-04	1.08E-03	1.23E-03	9.08E-04
4.73E-04	5.37E-04	3.96E-04	1.62E-03	1.84E-03	1.36E-03
9.45E-04	1.07E-03	7.92E-04	3.25E-03	3.69E-03	2.72E-03
1.89E-03	2.15E-03	1.58E-03	6.50E-03	7.38E-03	5.45E-03
4.10E-03	4.65E-03	3.43E-03	1.41E-02	1.60E-02	1.18E-02
8.19E-03	9.30E-03	6.86E-03	2.82E-02	3.20E-02	2.36E-02
1.23E-02	1.40E-02	1.03E-02	4.22E-02	4.80E-02	3.54E-02

Hazard Index

RfD = 0.000012 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.14	0.16	0.12
0.28	0.31	0.23
0.55	0.62	0.46
0.83	0.94	0.69
1.65	1.87	1.38
3.30	3.75	2.77
7.15	8.12	5.99
14.31	16.24	11.99
21.46	24.36	17.98
0.28	0.31	0.23
0.55	0.62	0.46
1.10	1.25	0.92
1.65	1.87	1.38
3.30	3.75	2.77
6.60	7.50	5.53
14.31	16.24	11.99
28.62	32.48	23.97
42.92	48.72	35.96
0.41	0.47	0.34
0.82	0.93	0.69
1.64	1.86	1.38
2.46	2.79	2.06
4.92	5.59	4.13
9.85	11.18	8.25
21.34	24.22	17.88
42.67	48.44	35.75
64.01	72.66	53.63

Lake Trout (All Sizes)

Tissue Concentration (85 +86)	ppm
Mean	0.17
Max.	0.61
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000003	0.000000
0.114	2	0.000625	0.000002	0.000005	0.000000
0.114	4	0.001249	0.000003	0.000011	0.000000
0.114	6	0.001874	0.000005	0.000016	0.000000
0.114	12	0.003748	0.000009	0.000033	0.000000
0.114	24	0.007496	0.000018	0.000065	0.000000
0.114	52	0.016241	0.000039	0.000142	0.000000
0.114	104	0.032482	0.000079	0.000283	0.000000
0.114	156	0.048723	0.000118	0.000425	0.000000
0.228	1	0.000625	0.000002	0.000005	0.000000
0.228	2	0.001249	0.000003	0.000011	0.000000
0.228	4	0.002499	0.000006	0.000022	0.000000
0.228	6	0.003748	0.000009	0.000033	0.000000
0.228	12	0.007496	0.000018	0.000065	0.000000
0.228	24	0.014992	0.000036	0.000131	0.000000
0.228	52	0.032482	0.000079	0.000283	0.000000
0.228	104	0.064964	0.000158	0.000566	0.000000
0.228	156	0.097447	0.000237	0.000849	0.000000
0.34	1	0.000932	0.000002	0.000008	0.000000
0.34	2	0.001863	0.000005	0.000016	0.000000
0.34	4	0.003726	0.000009	0.000032	0.000000
0.34	6	0.005589	0.000014	0.000049	0.000000
0.34	12	0.011178	0.000027	0.000097	0.000000
0.34	24	0.022356	0.000054	0.000195	0.000000
0.34	52	0.048438	0.000118	0.000422	0.000000
0.34	104	0.096877	0.000235	0.000844	0.000000
0.34	156	0.145315	0.000353	0.001266	0.000000

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
1.21E-05	4.35E-05	0.00E+00	4.17E-05	1.50E-04	0.00E+00
2.43E-05	8.71E-05	0.00E+00	8.34E-05	2.99E-04	0.00E+00
4.85E-05	1.74E-04	0.00E+00	1.67E-04	5.99E-04	0.00E+00
7.28E-05	2.61E-04	0.00E+00	2.50E-04	8.98E-04	0.00E+00
1.46E-04	5.23E-04	0.00E+00	5.01E-04	1.80E-03	0.00E+00
2.91E-04	1.05E-03	0.00E+00	1.00E-03	3.59E-03	0.00E+00
6.31E-04	2.26E-03	0.00E+00	2.17E-03	7.78E-03	0.00E+00
1.26E-03	4.53E-03	0.00E+00	4.34E-03	1.56E-02	0.00E+00
1.89E-03	6.79E-03	0.00E+00	6.51E-03	2.34E-02	0.00E+00
2.43E-05	8.71E-05	0.00E+00	8.34E-05	2.99E-04	0.00E+00
4.85E-05	1.74E-04	0.00E+00	1.67E-04	5.99E-04	0.00E+00
9.71E-05	3.48E-04	0.00E+00	3.34E-04	1.20E-03	0.00E+00
1.46E-04	5.23E-04	0.00E+00	5.01E-04	1.80E-03	0.00E+00
2.91E-04	1.05E-03	0.00E+00	1.00E-03	3.59E-03	0.00E+00
5.83E-04	2.09E-03	0.00E+00	2.00E-03	7.19E-03	0.00E+00
1.26E-03	4.53E-03	0.00E+00	4.34E-03	1.56E-02	0.00E+00
2.52E-03	9.06E-03	0.00E+00	8.68E-03	3.11E-02	0.00E+00
3.79E-03	1.36E-02	0.00E+00	1.30E-02	4.67E-02	0.00E+00
3.62E-05	1.30E-04	0.00E+00	1.24E-04	4.46E-04	0.00E+00
7.24E-05	2.60E-04	0.00E+00	2.49E-04	8.93E-04	0.00E+00
1.45E-04	5.20E-04	0.00E+00	4.98E-04	1.79E-03	0.00E+00
2.17E-04	7.79E-04	0.00E+00	7.47E-04	2.68E-03	0.00E+00
4.34E-04	1.56E-03	0.00E+00	1.49E-03	5.36E-03	0.00E+00
8.69E-04	3.12E-03	0.00E+00	2.99E-03	1.07E-02	0.00E+00
1.88E-03	6.75E-03	0.00E+00	6.47E-03	2.32E-02	0.00E+00
3.76E-03	1.35E-02	0.00E+00	1.29E-02	4.64E-02	0.00E+00
5.65E-03	2.03E-02	0.00E+00	1.94E-02	6.96E-02	0.00E+00

Hazard Index

RfD = 0.000012 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.06	0.23	0.00
0.13	0.45	0.00
0.25	0.91	0.00
0.38	1.36	0.00
0.76	2.72	0.00
1.52	5.44	0.00
3.29	11.79	0.00
6.57	23.59	0.00
9.86	35.38	0.00
0.13	0.45	0.00
0.25	0.91	0.00
0.51	1.81	0.00
0.76	2.72	0.00
1.52	5.44	0.00
3.03	10.89	0.00
6.57	23.59	0.00
13.15	47.18	0.00
19.72	70.76	0.00
0.19	0.68	0.00
0.38	1.35	0.00
0.75	2.71	0.00
1.13	4.06	0.00
2.26	8.12	0.00
4.52	16.23	0.00
9.80	35.18	0.00
19.61	70.35	0.00
29.41	105.53	0.00

Perch (All Sizes)

Tissue Concentration (85 +86)	ppm
Mean	0.02
Max.	0.04
Min.	0
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000000	0.000000
0.114	2	0.000625	0.000000	0.000000	0.000000
0.114	4	0.001249	0.000000	0.000001	0.000000
0.114	6	0.001874	0.000001	0.000001	0.000000
0.114	12	0.003748	0.000001	0.000002	0.000000
0.114	24	0.007496	0.000002	0.000004	0.000000
0.114	52	0.016241	0.000005	0.000009	0.000000
0.114	104	0.032482	0.000009	0.000019	0.000000
0.114	156	0.048723	0.000014	0.000028	0.000000
0.228	1	0.000625	0.000000	0.000000	0.000000
0.228	2	0.001249	0.000000	0.000001	0.000000
0.228	4	0.002499	0.000001	0.000001	0.000000
0.228	6	0.003748	0.000001	0.000002	0.000000
0.228	12	0.007496	0.000002	0.000004	0.000000
0.228	24	0.014992	0.000004	0.000009	0.000000
0.228	52	0.032482	0.000009	0.000019	0.000000
0.228	104	0.064964	0.000019	0.000037	0.000000
0.228	156	0.097447	0.000028	0.000056	0.000000
0.34	1	0.000932	0.000000	0.000001	0.000000
0.34	2	0.001863	0.000001	0.000001	0.000000
0.34	4	0.003726	0.000001	0.000002	0.000000
0.34	6	0.005589	0.000002	0.000003	0.000000
0.34	12	0.011178	0.000003	0.000006	0.000000
0.34	24	0.022356	0.000006	0.000013	0.000000
0.34	52	0.048438	0.000014	0.000028	0.000000
0.34	104	0.096877	0.000028	0.000055	0.000000
0.34	156	0.145315	0.000042	0.000083	0.000000

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
1.43E-06	2.86E-06	0.00E+00	4.91E-06	9.82E-06	0.00E+00
2.86E-06	5.71E-06	0.00E+00	9.82E-06	1.96E-05	0.00E+00
5.71E-06	1.14E-05	0.00E+00	1.96E-05	3.93E-05	0.00E+00
8.57E-06	1.71E-05	0.00E+00	2.94E-05	5.89E-05	0.00E+00
1.71E-05	3.43E-05	0.00E+00	5.89E-05	1.18E-04	0.00E+00
3.43E-05	6.85E-05	0.00E+00	1.18E-04	2.36E-04	0.00E+00
7.42E-05	1.48E-04	0.00E+00	2.55E-04	5.10E-04	0.00E+00
1.48E-04	2.97E-04	0.00E+00	5.10E-04	1.02E-03	0.00E+00
2.23E-04	4.45E-04	0.00E+00	7.66E-04	1.53E-03	0.00E+00
2.86E-06	5.71E-06	0.00E+00	9.82E-06	1.96E-05	0.00E+00
5.71E-06	1.14E-05	0.00E+00	1.96E-05	3.93E-05	0.00E+00
1.14E-05	2.28E-05	0.00E+00	3.93E-05	7.85E-05	0.00E+00
1.71E-05	3.43E-05	0.00E+00	5.89E-05	1.18E-04	0.00E+00
3.43E-05	6.85E-05	0.00E+00	1.18E-04	2.36E-04	0.00E+00
6.85E-05	1.37E-04	0.00E+00	2.36E-04	4.71E-04	0.00E+00
1.48E-04	2.97E-04	0.00E+00	5.10E-04	1.02E-03	0.00E+00
2.97E-04	5.94E-04	0.00E+00	1.02E-03	2.04E-03	0.00E+00
4.45E-04	8.91E-04	0.00E+00	1.53E-03	3.06E-03	0.00E+00
4.26E-06	8.52E-06	0.00E+00	1.46E-05	2.93E-05	0.00E+00
8.52E-06	1.70E-05	0.00E+00	2.93E-05	5.86E-05	0.00E+00
1.70E-05	3.41E-05	0.00E+00	5.86E-05	1.17E-04	0.00E+00
2.55E-05	5.11E-05	0.00E+00	8.78E-05	1.76E-04	0.00E+00
5.11E-05	1.02E-04	0.00E+00	1.76E-04	3.51E-04	0.00E+00
1.02E-04	2.04E-04	0.00E+00	3.51E-04	7.03E-04	0.00E+00
2.21E-04	4.43E-04	0.00E+00	7.61E-04	1.52E-03	0.00E+00
4.43E-04	8.86E-04	0.00E+00	1.52E-03	3.04E-03	0.00E+00
6.64E-04	1.33E-03	0.00E+00	2.28E-03	4.57E-03	0.00E+00

	Hazard Index	
RfD =	0.000012 Liver	
Mean Conc.	Max. Conc.	Min. Conc.
0.01	0.01	0.00
0.01	0.03	0.00
0.03	0.06	0.00
0.04	0.09	0.00
0.09	0.18	0.00
0.18	0.36	0.00
0.39	0.77	0.00
0.77	1.55	0.00
1.16	2.32	0.00
0.01	0.03	0.00
0.03	0.06	0.00
0.06	0.12	0.00
0.09	0.18	0.00
0.18	0.36	0.00
0.36	0.71	0.00
0.77	1.55	0.00
1.55	3.09	0.00
2.32	4.64	0.00
0.02	0.04	0.00
0.04	0.09	0.00
0.09	0.18	0.00
0.13	0.27	0.00
0.27	0.53	0.00
0.53	1.06	0.00
1.15	2.31	0.00
2.31	4.61	0.00
3.46	6.92	0.00

Walleye (10 - 20 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.02
Max.	0.08
Min.	0
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000000	0.000000
0.114	2	0.000625	0.000000	0.000001	0.000000
0.114	4	0.001249	0.000000	0.000001	0.000000
0.114	6	0.001874	0.000001	0.000002	0.000000
0.114	12	0.003748	0.000001	0.000004	0.000000
0.114	24	0.007496	0.000002	0.000009	0.000000
0.114	52	0.016241	0.000005	0.000019	0.000000
0.114	104	0.032482	0.000009	0.000037	0.000000
0.114	156	0.048723	0.000014	0.000056	0.000000
0.228	1	0.000625	0.000000	0.000001	0.000000
0.228	2	0.001249	0.000000	0.000001	0.000000
0.228	4	0.002499	0.000001	0.000003	0.000000
0.228	6	0.003748	0.000001	0.000004	0.000000
0.228	12	0.007496	0.000002	0.000009	0.000000
0.228	24	0.014992	0.000004	0.000017	0.000000
0.228	52	0.032482	0.000009	0.000037	0.000000
0.228	104	0.064964	0.000019	0.000074	0.000000
0.228	156	0.097447	0.000028	0.000111	0.000000
0.34	1	0.000932	0.000000	0.000001	0.000000
0.34	2	0.001863	0.000001	0.000002	0.000000
0.34	4	0.003726	0.000001	0.000004	0.000000
0.34	6	0.005589	0.000002	0.000006	0.000000
0.34	12	0.011178	0.000003	0.000013	0.000000
0.34	24	0.022356	0.000006	0.000026	0.000000
0.34	52	0.048438	0.000014	0.000055	0.000000
0.34	104	0.096877	0.000028	0.000111	0.000000
0.34	156	0.145315	0.000042	0.000166	0.000000

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
1.43E-06	5.71E-06	0.00E+00	4.91E-06	1.96E-05	0.00E+00
2.86E-06	1.14E-05	0.00E+00	9.82E-06	3.93E-05	0.00E+00
5.71E-06	2.28E-05	0.00E+00	1.96E-05	7.85E-05	0.00E+00
8.57E-06	3.43E-05	0.00E+00	2.94E-05	1.18E-04	0.00E+00
1.71E-05	6.85E-05	0.00E+00	5.89E-05	2.36E-04	0.00E+00
3.43E-05	1.37E-04	0.00E+00	1.18E-04	4.71E-04	0.00E+00
7.42E-05	2.97E-04	0.00E+00	2.55E-04	1.02E-03	0.00E+00
1.48E-04	5.94E-04	0.00E+00	5.10E-04	2.04E-03	0.00E+00
2.23E-04	8.91E-04	0.00E+00	7.66E-04	3.06E-03	0.00E+00
2.86E-06	1.14E-05	0.00E+00	9.82E-06	3.93E-05	0.00E+00
5.71E-06	2.28E-05	0.00E+00	1.96E-05	7.85E-05	0.00E+00
1.14E-05	4.57E-05	0.00E+00	3.93E-05	1.57E-04	0.00E+00
1.71E-05	6.85E-05	0.00E+00	5.89E-05	2.36E-04	0.00E+00
3.43E-05	1.37E-04	0.00E+00	1.18E-04	4.71E-04	0.00E+00
6.85E-05	2.74E-04	0.00E+00	2.36E-04	9.42E-04	0.00E+00
1.48E-04	5.94E-04	0.00E+00	5.10E-04	2.04E-03	0.00E+00
2.97E-04	1.19E-03	0.00E+00	1.02E-03	4.08E-03	0.00E+00
4.45E-04	1.78E-03	0.00E+00	1.53E-03	6.13E-03	0.00E+00
4.26E-06	1.70E-05	0.00E+00	1.46E-05	5.86E-05	0.00E+00
8.52E-06	3.41E-05	0.00E+00	2.93E-05	1.17E-04	0.00E+00
1.70E-05	6.81E-05	0.00E+00	5.86E-05	2.34E-04	0.00E+00
2.55E-05	1.02E-04	0.00E+00	8.78E-05	3.51E-04	0.00E+00
5.11E-05	2.04E-04	0.00E+00	1.76E-04	7.03E-04	0.00E+00
1.02E-04	4.09E-04	0.00E+00	3.51E-04	1.41E-03	0.00E+00
2.21E-04	8.86E-04	0.00E+00	7.61E-04	3.04E-03	0.00E+00
4.43E-04	1.77E-03	0.00E+00	1.52E-03	6.09E-03	0.00E+00
6.64E-04	2.66E-03	0.00E+00	2.28E-03	9.13E-03	0.00E+00

```
=====================================
            Hazard Index
      RfD =    0.000012 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
=====================================

        0.01        0.03        0.00
        0.01        0.06        0.00
        0.03        0.12        0.00
        0.04        0.18        0.00
        0.09        0.36        0.00
        0.18        0.71        0.00
        0.39        1.55        0.00
        0.77        3.09        0.00
        1.16        4.64        0.00

        0.01        0.06        0.00
        0.03        0.12        0.00
        0.06        0.24        0.00
        0.09        0.36        0.00
        0.18        0.71        0.00
        0.36        1.43        0.00
        0.77        3.09        0.00
        1.55        6.19        0.00
        2.32        9.28        0.00

        0.02        0.09        0.00
        0.04        0.18        0.00
        0.09        0.35        0.00
        0.13        0.53        0.00
        0.27        1.06        0.00
        0.53        2.13        0.00
        1.15        4.61        0.00
        2.31        9.23        0.00
        3.46       13.84        0.00
```

Whitefish (10 - 20 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.09
Max.	0.13
Min.	0.05
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000001	0.000000
0.114	2	0.000625	0.000001	0.000001	0.000000
0.114	4	0.001249	0.000002	0.000002	0.000001
0.114	6	0.001874	0.000002	0.000003	0.000001
0.114	12	0.003748	0.000005	0.000007	0.000003
0.114	24	0.007496	0.000010	0.000014	0.000005
0.114	52	0.016241	0.000021	0.000030	0.000012
0.114	104	0.032482	0.000042	0.000060	0.000023
0.114	156	0.048723	0.000063	0.000090	0.000035
0.228	1	0.000625	0.000001	0.000001	0.000000
0.228	2	0.001249	0.000002	0.000002	0.000001
0.228	4	0.002499	0.000003	0.000005	0.000002
0.228	6	0.003748	0.000005	0.000007	0.000003
0.228	12	0.007496	0.000010	0.000014	0.000005
0.228	24	0.014992	0.000019	0.000028	0.000011
0.228	52	0.032482	0.000042	0.000060	0.000023
0.228	104	0.064964	0.000084	0.000121	0.000046
0.228	156	0.097447	0.000125	0.000181	0.000070
0.34	1	0.000932	0.000001	0.000002	0.000001
0.34	2	0.001863	0.000002	0.000003	0.000001
0.34	4	0.003726	0.000005	0.000007	0.000003
0.34	6	0.005589	0.000007	0.000010	0.000004
0.34	12	0.011178	0.000014	0.000021	0.000008
0.34	24	0.022356	0.000029	0.000042	0.000016
0.34	52	0.048438	0.000062	0.000090	0.000035
0.34	104	0.096877	0.000125	0.000180	0.000069
0.34	156	0.145315	0.000187	0.000270	0.000104

Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* = 55 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
6.43E-06	9.28E-06	3.57E-06	2.21E-05	3.19E-05	1.23E-05
1.29E-05	1.86E-05	7.14E-06	4.42E-05	6.38E-05	2.45E-05
2.57E-05	3.71E-05	1.43E-05	8.83E-05	1.28E-04	4.91E-05
3.86E-05	5.57E-05	2.14E-05	1.33E-04	1.91E-04	7.36E-05
7.71E-05	1.11E-04	4.28E-05	2.65E-04	3.83E-04	1.47E-04
1.54E-04	2.23E-04	8.57E-05	5.30E-04	7.66E-04	2.94E-04
3.34E-04	4.83E-04	1.86E-04	1.15E-03	1.66E-03	6.38E-04
6.68E-04	9.65E-04	3.71E-04	2.30E-03	3.32E-03	1.28E-03
1.00E-03	1.45E-03	5.57E-04	3.45E-03	4.98E-03	1.91E-03
1.29E-05	1.86E-05	7.14E-06	4.42E-05	6.38E-05	2.45E-05
2.57E-05	3.71E-05	1.43E-05	8.83E-05	1.28E-04	4.91E-05
5.14E-05	7.42E-05	2.86E-05	1.77E-04	2.55E-04	9.82E-05
7.71E-05	1.11E-04	4.28E-05	2.65E-04	3.83E-04	1.47E-04
1.54E-04	2.23E-04	8.57E-05	5.30E-04	7.66E-04	2.94E-04
3.08E-04	4.45E-04	1.71E-04	1.06E-03	1.53E-03	5.89E-04
6.68E-04	9.65E-04	3.71E-04	2.30E-03	3.32E-03	1.28E-03
1.34E-03	1.93E-03	7.42E-04	4.59E-03	6.64E-03	2.55E-03
2.00E-03	2.90E-03	1.11E-03	6.89E-03	9.95E-03	3.83E-03
1.92E-05	2.77E-05	1.06E-05	6.59E-05	9.51E-05	3.66E-05
3.83E-05	5.54E-05	2.13E-05	1.32E-04	1.90E-04	7.32E-05
7.66E-05	1.11E-04	4.26E-05	2.63E-04	3.81E-04	1.46E-04
1.15E-04	1.66E-04	6.39E-05	3.95E-04	5.71E-04	2.20E-04
2.30E-04	3.32E-04	1.28E-04	7.90E-04	1.14E-03	4.39E-04
4.60E-04	6.64E-04	2.55E-04	1.58E-03	2.28E-03	8.78E-04
9.96E-04	1.44E-03	5.54E-04	3.43E-03	4.95E-03	1.90E-03
1.99E-03	2.88E-03	1.11E-03	6.85E-03	9.90E-03	3.81E-03
2.99E-03	4.32E-03	1.66E-03	1.03E-02	1.48E-02	5.71E-03

Hazard Index
RfD = 0.000012 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.03	0.05	0.02
0.07	0.10	0.04
0.13	0.19	0.07
0.20	0.29	0.11
0.40	0.58	0.22
0.80	1.16	0.45
1.74	2.51	0.97
3.48	5.03	1.93
5.22	7.54	2.90
0.07	0.10	0.04
0.13	0.19	0.07
0.27	0.39	0.15
0.40	0.58	0.22
0.80	1.16	0.45
1.61	2.32	0.89
3.48	5.03	1.93
6.96	10.05	3.87
10.44	15.08	5.80
0.10	0.14	0.06
0.20	0.29	0.11
0.40	0.58	0.22
0.60	0.86	0.33
1.20	1.73	0.67
2.40	3.46	1.33
5.19	7.50	2.88
10.38	14.99	5.77
15.57	22.49	8.65

CHAPTER 7

HAZARD ASSESSMENT AND THE CHARACTERIZATION OF CARCINOGENIC AND OTHER RISKS TO HUMANS EXPOSED TO CHLORDANE

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INTRODUCTION

Chlordane is a cyclodiene insecticide with persistent, bioaccumulative properties causing the chemical to be widely distributed in the environment. Chlordane was registered for use as a pesticide from 1948 to 1988. Subterranean termite control was the only allowable use for chlordane from 1983 to April 1988.

Technical chlordane is a mixture of compounds containing 19% cis-chlordane, 24% trans-chlordane, 7% trans-nonachlor, 10% hepatchlor, 21% chlordene isomers, and 18% miscellaneous chlorinated products (U.S. EPA, 1985). A higher purity formulation of chlordane was also manufactured by Velsicol Chemical Corporation, HCS-3260, containing 74% cis- and 24% trans-chlordane.

Chemical and Physical Properties

The water solubility of technical chlordane is 9 ug/L at 25°C. The solubility of cis:trans 3:1 chlordane is 56 ug/L at 25°C. The evaporation half-life (t1/2) of the cis- and trans-isomers from water at a depth of one meter is 28 hours at 25° and 33 hours at 25°, respectively. A log octanol/water partition coefficient of 3.32 has been reported for chlordane (U.S. EPA, 1986). The sediment-water sorption coefficient value for chlordane is 1.4×10^{-5} (U.S. EPA, 1985). Evaporation, sorption and bioaccumulation are probably the three most important fate-determining processes for chlordane constituents (U.S. EPA, 1985).

Human Exposure

The long-term use of chlordane in agriculture, for turf treatment, garden, and household use, and for soil treatment around buildings has resulted in the presence of chlordane residues in foods, soils, sediments, wildlife, and in air near application sites. Excluding exposures related to pesticide application, the consumption of contaminated food is the most important environmental route of exposure to chlordane in the United States.

Air

Ambient air is considered to be an insignificant source of chlordane exposure (WHO, 1984). A recent study of urban air in Bloomington, Indiana measured a mean chlordane concentration of 11 ng/m^3 with a range of 0.8 to 49 ng/m^3 (Anderson and Hites, 1988). On the other hand, pesticide applications have contributed significantly to local air concentrations of chlordane. Indoor air concentrations from < 1 to 610,000 ng/m^3 have been reported related to termite control. Chlordane concentrations as high as 72 ppm have been found in house dust for up to four years after fumigation treatment.

Water

Chlordane has been detected in surface water, groundwater, and drinking water generally in ng/L concentrations. Drinking water is considered to be a relatively insignificant source of chlordane (WHO, 1984).

Food

Data for adult market basket samples collected and analyzed between October 1980 and March 1982 were reported by Gartrell et al. (1986b). Chlordane concentrations in all food groups ranged between trace to 2 ppb. Average daily intake of chlordane and octachlor epoxide from food was estimated to be 0.079 and 0.213 ug/day. Chlordane was identified in potatoes and garden fruits while oxtachlor epoxide was detected in dairy products, meat, fish, and poultry, potatoes, garden fruits and other fruits. The highest percent of the average daily intake was accounted for by meat, fish and poultry (0.169 ug/day, 79%), followed by dairy products (0.038 ug/day, 17.7%). Average daily intake of trans-nonachlor, which was detected in meat, fish, and poultry, and garden fruits was 0.078 ug/day. The total intake of chlordane and trans-nonachlor in 1981/82 was 0.004 ug/kg/day and 0.001 ug/kg/day, respectively. These levels had not changed since 1978.

Pesticide residues in the food of infants (six months old) and toddlers (two years old) were reported for the same time period (Gartrell et al., 1986a). Chlordane was not identified in the infant diet, but octachlor epoxide was identified in whole milk (31.6%), and meat, fish and poultry (68%). The average daily intake for all food was reported to be 0.018 ug/day. Chlordane was found in the toddler diet in oils and fats (0.005 ug/day). Octachlor epoxide was found in whole milk, other dairy products, and meat, fish and poultry. The average daily intake for all food was 0.038 ug/day. The food category, meat, fish and poultry, was the major contributor of chlordane to the toddler diet accounting for 52.7% of the total daily intake (0.02 ug/day). Whole milk (0.0042 ug/day) and other dairy products (0.013 ug/day) accounted for 11% and 35.3%, respectively. Trans-nonachlor was not reported in the diets of infants or toddlers.

The daily chlordane intake was equivalent to an exposure of 0.002 ug/kg/day and 0.003 ug/kg/day in 8.2 kg infants and 13.7 kg toddlers, respectively. The daily chlordane dose (ug/kg/day) in these age groups had decreased by approximately 80 - 90% since 1978.

Human Body Burden

Chlordane residues are widely dispersed in the United States population. Oxychlordane was detected in human adipose tissue in 95% of 785 samples collected from 1976 to 1980. Concentrations ranged between not-detected to 10 ppb (Murphy et al., 1983). Oxychlordane was measured in 4% of 4200 serum samples taken from the United States population during the same time period and levels ranged from not-detected to 1 ppb.

PHARMACODYNAMICS OF CHLORDANE

HUMAN

Absorption and Distribution

Chlordane residues have been identified in the blood of pesticide control operators, pesticide manufacturers, and the general population reflecting high occupational exposure and widespread environmental contamination. Chlordane concentrations in human tissues are related to the source, degree, and duration of exposure to chlordane.

A total of 51 male workers aged 21 to 64 years (mean 34 years) with chlordane spraying experience in eight pest control companies and 19 male workers aged 18 to 67 years (mean 35 years) with no chlordane spraying experience were studied by Saito et al. (1986). The duration of employment was similar in both groups (0.5 to 21 years, mean 6.1 years and 0.4 to 22.8 years, mean 6.5 years). Trans-nonachlor, oxychlordane, and heptachlor epoxide were detected in the blood of 37%, 22%, and 20% of the exposed group. Trans- and cis-chlordane were below the detectable level of 0.3 ng/g in the blood of the pesticide sprayers. Mean concentrations of trans-nonachlor, oxychlordane, and heptachlor epoxide were 0.55 ppb (N.D. - 2.9 ppb), 0.29 ppb (N.D. - 1.5 ppb), and 0.29 ppb (N.D. - 1.6 ppb). Chlordane residues were below the level of detection in the nonexposed group.

The association of total chlordane residues in blood with various exposure parameters was evaluated in 19 workers sprayers with detectable chlordane levels. Total chlordane in blood was not correlated with age or duration of employment. Concentrations were highly correlated ($p<0.001$) with the total amount of chlordane sprayed ($r=0.68$), and the number of spraying days, particularly in the last three months ($r=0.81$), but also in the last six ($r=0.75$) and twelve months ($r=0.78$). The mean concentration of total chlordane in the blood of these workers was 2.2 ± 1.9 ppm.

The mean duration of employment was significantly different between these 19 workers compared to 32 chlordane sprayers who had no detectable blood chlordane levels (8.8 ± 5.4 years versus 4.4 ± 4.1 years, $p<0.01$). A significantly greater amount of 40% emulsifiable concentrate was sprayed by those with detectable blood chlordane concentrations compared to workers with nondetectable levels (179 ± 26 versus 45 ± 34 liters/year). The number of spraying days in the last three, six and twelve months was also significantly greater. Respirator usage appeared to be associated with increased chlordane blood levels. The authors suggested that respirator users sprayed larger amounts of the pesticide and experienced greater dermal exposure.

Total chlordane residues (trans-nonachlor plus oxychlordane) in the plasma of seven pesticide spraymen from one firm were correlated with duration of employment ($r=0.83$, $p<0.05$), although the relationship was not evident in the five and three workers from two other firms (Takamiya, 1987). Levels of trans-nonachlor were also related to length of employment in the same seven workers ($r=0.88$, $p<0.01$) while oxychlordane levels were not related ($r=0.34$). The relative amount of termaticide used by the firms was not known, and except for five persons, exposure periods were less than 10 years among the 22 to 42 year old workers.

The concentration of trans-nonachlor in eleven workers employed more than five years was higher than the levels in workers employed for less than three years (3.7 ± 3.6 ppb versus 0.98 ± 0.65 ppb, $p<0.01$). The concentration of oxychlordane in both groups was 1.4 ppb. Oxychlordane and trans-nonachlor levels were correlated ($r=0.92$, $p<0.01$) in the persons exposed for a longer time period suggesting a similar pattern of accumulation in humans for the two compounds. The proportion of total chlordane comprised by trans-nonachlor in blood increased with time and reached a plateau at 60 to 80 percent. Cis- and trans-chlordane were not detected in the 19 workers.

Trans-nonachlor and oxychlordane were also the most prevalent components of the chlordane residues identified in the blood of 43 representatives of the Japanese general population (Wariishi et al., 1986). Blood specimens were obtained from 22 male and 21 female healthy medical students, researchers, and office workers of the University of Tokushima. Geometric mean concentrations (ng/ml) in the blood of males were 0.2 ± 1.9, 0.09 ± 2.1, 0.09 ± 2.2, 0.05 ± 1.8, 0.03 ± 1.7 for oxychlordane, trans-nonachlor, trans-chlordane, cis-chlordane, and cis-nonachlor, respectively. Female blood contained 0.23 ± 1.8, 0.07 ± 1.9, 0.11 ± 1.6, 0.06 ± 1.6, and 0.03 ± 2.0 ng/ml of the same chlordane constituents and metabolites. Total chlordane residues in blood were 0.51 ng/ml (ppb) and 0.46 ng/ml (ppb) for males and females, respectively. Heptachlor epoxide was also measured, but concentrations were not reported.

An evaluation of this population for potential sources of chlordane exposure revealed that those living in residences treated for termites within the past five years (N = 7) had significantly higher concentrations of oxychlordane and trans-nonachlor than those who did not (N = 24). Those reporting frequent consumption of fish (N = 11) had significantly higher trans-nonachlor concentrations than those with a low frequency of fish consumption (N = 12). Close proximity to areas contaminated by agricultural chemical application or frequency of meat intake were not related to blood concentrations of the compounds.

Breast milk concentrations were measured in 29 healthy individuals, aged 19 to 34 years, residing in the same geographic area in Japan (Tojo et al., 1986). Samples were taken before or during the breastfeeding of the mothers' three month old infants. Chlordane constituents were present in the breast milk in the following decreasing order of concentration: heptachlor epoxide, trans-nonachlor, oxychlordane and cis-nonachlor. Geometric mean concentrations in whole milk (ng/ml) were 0.55 ± 2.24 for trans-nonachlor, 0.39 ± 1.86 for oxychlordane, 0.04 ± 1.59 for trans-chlordane, 0.09 ± 2.27 for cis-chlordane, 0.14 ± 1.97 for cis-nonachlor, and 0.66 ± 1.62 for heptachlor epoxide. Quantitated on a fat basis, these concentrations (ng/g) were 15.7 ± 1.9, 11.5 ± 1.7, 1.2 ± 2.1, 3.08 ± 2.5, 4.0 ± 4.6, and 20.0 ± 1.4, respectively. The levels of these compounds were positively correlated with the lipid content of milk.

Individuals who reported a lower frequency of meat consumption (N = 3) had significantly higher concentrations of trans-nonachlor, oxychlordane, and cis-nonachlor in breast milk than individuals reporting high meat consumption (N =

8). Total chlordane concentrations in low frequency meat eaters were 5.13 ng/ml compared to 2.0 ng/ml in those eating more meat ($p<0.01$). The frequency of fish intake, proximity to agricultural pesticide use, or residential pest control had no effect on chlordane residues in breast milk. Although not statistically significant, the level of trans-nonachlor, oxychlordane, cis-chlordane, and heptachlor epoxide were higher in those eating more fish. The authors suggested that low meat consumption may represent high fish consumption.

A study of human milk samples collected from women in several maternity hospitals in the Tokyo metropolitan area from September 1978 to November 1979 found levels of oxychlordane and trans-nonachlor in 100% of the samples (Miyazaki et al., 1980). Eleven whole milk samples taken in 1978 contained oxychlordane ranging between 0.1 and 1.0 ppb with a mean of 0.48 ppb and twelve samples taken in 1979 ranged between 0.3 and 1.1 ppb with a mean of 0.52 ppb. Trans-nonachlor concentrations ranged between 0.1 and 0.9 ppb with a mean of 0.54 in 1978 and between 0.5 and 1.1 ppb with a mean of 0.71 ppb in 1979. Cis-nonachlor was also detected in pooled 1978 and 1979 samples at a concentration of 0.06 and 0.18 ppb, respectively. Samples collected in 1979 also contained 0.16 ppb trans-chlordane and 0.13 ppb cis-chlordane. The authors concluded that the presence of chlordane residues in human breast milk indicates environmental pollution as the source of exposure.

Chlordane residues are ubiquitous in mother's milk in the United States. Savage et al. (1981) conducted a national study of chlorinated hydrocarbon insecticide residues in human milk in the United States and found that 74% of the 1436 milk samples contained detectable levels of oxychlordane (> 1 ppb) while chlordane was not identified at a limit of detection of 30 ppb.

Seventy-two percent of the 378 samples collected in the Midwest contained detectable levels of oxychlordane. Samples that contained 51 to 100 ppb (fat adjusted) oxychlordane comprised 40.7% of the total U.S. sample while 44.8% of midwestern samples were in this range. Mean oxychlordane concentrations in the total U.S. and Midwest samples were 95.8 and 80.6 ppb (fat adjusted), respectively.

The identification of oxychlordane in significant amounts in human blood and breast milk indicates that humans have at least one metabolic pathway in common with experimental animals. On the other hand, nonachlor, not a common storage product in rodents, does not appear to be easily metabolized by humans. Both compounds are not easily eliminated from the human body. The correlation of concentrations of these compounds with the fat content in breast milk suggests that oxychlordane and trans-nonachlor accumulate in adipose tissue, in a manner similar to the distribution pattern reported for other mammals.

Elimination

The transfer of chlordane residues in breast milk to nursing infants is potentially a significant source of elimination in human females. Mes et al. (1984) followed pesticide residue levels in the breast milk and blood from 16 donors over the course of a three month lactation period. Oxychlordane levels in milk began at 1.2 ng/g whole milk at seven days postpartum, increased to 1.6 ng/g during the first 30 days, then decreased to 1.3 ng/g at 98 days after parturition. Levels of trans-nonachlor, which decreased only slightly if at all, were 1.2 ng/g whole milk at seven days post-partum and 1.1 ng/g whole milk at 98 days after birth. The changes were not statistically significant. The concentration in whole blood was 0.2 ng/g seven days after parturition, with a high of 0.5 ng/g at 56 days, and was not detectable at 98 days postpartum. The percent fat in blood also decreased over the three months, however. The ratio of oxychlordane whole milk levels compared to whole blood residues during the first 70 days of lactation when concentrations were detected in maternal blood averaged around 9, ranging between 2 and 20. The authors estimated that oxychlordane concentrations would rise to approximately 0.08 ug/g body fat in the body of an infant nursing for 14 weeks.

Data concerning the excretion of chlordane residues in humans have been collected from poisoning incidents where individuals ingested chlordane formulations. One incident involved a 20 month old, 12.7 kg white boy who drank 74% technical chlordane (Curley and Garrettson, 1969). The concentration in blood 30 minutes after convulsions stopped was 2.71 mg/liter (ppm). The concentration in fat at this time was 3.12 mg/kg body weight and was reported to rise rapidly over the next 48 hours. A maximum fat concentration occurred at eight days. The concentration in fat had decreased only slightly after 94 days. The ratio of fat to serum concentration on the eighth day was 147:1 and was 1470:1 at three months. At this point fat and serum concentrations were 25.53 mg/kg body weight and 0.017 mg/liter, respectively. The chlordane concentration in urine collected during the first 24 hours was 0.309 mg/liter. A half-life of 21 days was estimated from data on serum levels taken at day 8 and day 94.

A report of chlordane intoxication by investigators who followed an individual for 49 days calculated a terminal elimination half-life of 34 days from whole blood concentrations three to 49 days post-ingestion (Olanoff et al., 1983). The pattern of pesticide concentration in the patient's blood suggested a two-phase compartmental model of disposition. Peak chlordane levels of 5 mg/L were measured in whole blood 3.5 hours after ingestion. The whole blood chlordane concentration at 49 days was 39.04 ppb, 38% of the chlordane levels measured on day three. On the 58th day after the incident, concentrations of oxychlordane, trans-nonachlor, and heptachlor epoxide in the liver were 44.8, 50.8, and 455.8 ppb (wet weight), respectively. Concentrations of these three chlordane residues in the abdominal fat of the patient were 510, 1,880, and 2,620 ppb, respectively. No chlordane residues were detected in intermittent urine specimens collected at 12 to 24 hour intervals beginning 15 hours after the incident.

A biological half-life of 88 days was calculated from the decline in serum chlordane concentrations in a child, four years old, who had ingested a 45% emulsifiable concentrate in 1968 (Aldrich and Holmes, 1969). This child was followed for 130 days after the incident and the half-life was calculated from the data taken during the last 90 days. Chlordane concentrations in serum fell from 3.4 to 0.138 ppm during the first three days, and were 0.03 ppm at 130 days. Concentrations in urine decreased from 1.93 to 0.05 ppm during the first three days, rose to 0.13 ppm 35 days later, and then decreased again. Fecal chlordane levels were 719 and 105 ppm on the second and third days after the chlordane was ingested. Chlordane was not detected in feces after one or two months.

ANIMAL

Absorption

Little data are available concerning the absorption of chlordane in orally exposed animals. An absorption fraction of 82.9% was estimated based on a comparison of the area under the curve of chlordane concentration in venous blood during 48 hours after an oral injection or an intravenous injection of

100 ug/kg trans-chlordane in male Wistar strain rats, two per group (Ohno et al., 1986). A similar absorption fraction was calculated from rats (two per dose route) dosed orally or intravenously with 1 mg/kg trans-chlordane.

The curves were fitted to a two compartment model. The concentration in venous blood after an oral administration reached a maximum at four hours. The half-life of elimination from blood calculated from intravenously dosed animals was 0.8 hour in the rapid phase and 36.9 hours in the slow phase for rats dosed with 100 ug/kg/day. The half-lives were similar for rats in the high dose group. Chlordane residues in the intestine of orally dosed animals decreased rapidly comprising 4 - 5% of the administered dose one day after administration and 0.8 - 1.0% on the second day after dosing. Chlordane was therefore rapidly absorbed and/or eliminated from the intestine.

The absorption of chlordane appears to be slower in mice compared to rats. Ewing et al. (1985) compared blood concentrations for 168 hours after male Sprague-Dawley rats and male C57BL/6JX mice were given a single oral dose of 1 mg/kg cis-chlordane. Absorption was similar during the first two hours after administration except that residues were higher in the rats. Blood levels in the mice increased with time, however. Peak blood levels of 81 ng/ml (ppb) occurred at two hours in the rats, whereas a maximum level of 113 ng/ml was observed in the blood of the mice at eight hours.

Distribution

Distribution was studied in Sprague-Dawley rats dosed by gavage with 0.05, 0.2, and 1.0 mg/kg HCS-3260-^{14}C, a 3:1 mixture of cis- and trans-chlordane (Barnett and Dorough, 1974). At one and seven days after administration, chlordane residues were detected in several tissues with highest levels in adipose tissue. Tissues accumulated residues in the following order of decreasing concentration; fat, liver, kidney, brain and muscle. Chlordane levels were highest on day one and decreased during the week after dosing. Concentrations in all sampled tissues increased in relation to dose level. The levels in the adipose tissue of female rats were higher than those detected in males. Adipose tissue concentrations in males one day after a single dose of 0.05, 0.2, or 1.0 mg/kg were 0.09, 0.35, and 2.58 ppm, respectively. Adipose tissue concentrations in females in the same three dose groups were 0.14, 0.5, and 3.71 ppm, respectively. In contrast, residues in the brain, muscle, liver and kidney of males were higher than in females.

The accumulation of ^{14}C residues in animal tissues was not equivalent when either a single 0.2 mg/kg dose of cis- or trans-chlordane was administered. Trans-chlordane residues accumulated to a greater degree than cis-chlordane residues. At one day after dosing, concentrations of cis-chlordane residues in adipose tissue were 0.32 and 0.21 ppm in females and males, respectively. Concentrations of trans-chlordane were 0.66 and 0.51 ppm in females and males, respectively, about twice as much as the cis-isomer. The pattern of distribution of both isomers was similar to the HCS-3260 mixture. The chlordane metabolite, oxychlordane, when administered to female rats at a single dose of 0.2 mg/kg, did not decrease to the same extent as HCS-3260 or the isomers after seven days. Concentrations at one and seven days after oxychlordane administration were 0.54 and 0.5 ppm, respectively.

Trans-chlordane residues accumulated to a greater extent (1.5 times in fat) than cis-chlordane in the tissues of female rats fed 25 ppm for 14 days. The rate of decrease in residue levels was not considered to be different for the two isomers.

Maximum concentrations were attained in the liver (21 ug/g), kidney (18.3 ug/g), brain (3.44 ug/g), muscle (1.43 ug/g), and blood (0.658 ug/ml) of male Wistar rats four hours after an oral administration of 10 mg/kg trans-chlordane (Ohno et al., 1986). Skin and adipose tissue accumulated maximum concentrations after 8 (5.06 ug/g) and 16 hours (11.1 ug/g), respectively. In animals receiving 50 ug/kg, maximum concentrations were achieved in the kidney (0.0698 ug/g), brain (0.014 ug/g), muscle (0.00832 ug/g), and blood (0.00334 ng/ml) four hours after an oral dose. Chlordane residues reached a high of 0.107 ug/g in the liver after two hours. The highest levels were reached in the skin (0.027 ug/g) and adipose tissue (0.0935 ug/g) after 8 hours and four days, respectively. Tissue concentrations and the rate of accumulation, therefore, are related to dose level. At seven days after dosing with 10 mg/kg, tissues contained residues in the following decreasing order of concentration; adipose tissue, liver, kidney, skin, muscle, brain, and blood. The relative order for tissue concentration in animals given 50 ug/kg was adipose tissue, kidney, liver, skin, brain, blood, and muscle.

Sprague-Dawley rats and C57BL/6JX mice accumulated maximum concentrations in liver, fat, kidney, and lungs in order of decreasing concentration (Ewing et al., 1985). Mice differed from rats in that maximum concentrations in muscle and testes were higher than the brain in mice, while the maximum levels in the brain of rats were higher than those in testes or muscle. Peak levels occurred at four hours for all tissues in mice, and several tissues in rats, except for brain, liver, and muscle which accumulated maximum concentrations after two hours. The peak concentrations were higher in rats for all tissues except muscle. Maximum concentrations in rat tissues compared to mouse tissues were 1.5 times higher in fat, 1.7 times higher in the liver, 2.1 times higher in the kidney, 3.3 times higher in the brain, and 1.5 times higher in the testes.

Tissue to blood ratios over the initial 24 hours after chlordane administration are presented in Table 1. Ratios were higher in the rat for all tissues except lungs or muscle. The higher levels in the rat reflect the relatively higher residue levels in the tissues of the rat and the lower blood concentrations in the rat after the first four hours. The rat cleared chlordane residues from the blood faster than the mouse. The rat cleared 50% of the maximum concentration from the blood in approximately ten hours, while clearance of the same proportion from the blood of the mouse was accomplished in about 18 hours.

The authors concluded that since peak concentrations occurred in the blood and tissues in 2 or 4 hours in both the rat and mouse, the materials absorbed during the first two hours and stored in tissues were probably the same. Since the higher blood concentrations in the mouse did not increase tissue concentrations after two hours, the materials absorbed during this time had possibly been altered in the gut prior to absorption and were not accumulated within the body.

Table 1. A Comparison of the Tissue to Blood Ratio for Various Tissues of the Rat and Mouse During the First 24 Hours After a Single Oral Dose of 1.0 mg/kg Cis-Chlordane.

	0 - 24 Hour Tissue/Blood Ratio	
Tissue	Rats	Mice
Brain	1.7	0.6
Liver	17.6	11.0
Kidney	8.1	3.2
Lungs	2.6	3.0
Testes	1.6	0.9
Muscle	1.2	1.9
Fat	19.8	8.8

The relative accumulation of residues in the tissues of male and female rats fed 1, 5, or 25 ppm HCS-3260 in the diet for 56 days was similar to the distribution of the mixture after a single dose (Barnett and Dorough, 1974). Chlordane-^{14}C equivalent concentrations in fat were 3 - 4 times the dietary concentration at 56 days. Male rats accumulated residues to a lesser degree than females. Male rats fed 25 ppm HCS-3260 for 56 days had 15 ppm chlordane-^{14}C in fat while females fed the same concentration had 20 ppm. A steady state had not been reached at 56 days in any of the dose groups.

Rabbits, four males, were given four doses of cis- or trans-chlordane (approximately 100 mg/capsule) at four day intervals (Balba and Saha, 1978). The animals were killed on the fifth day after the fourth dose. The pattern of distribution differed for the two isomers. The levels of cis- residues were in the following decreasing order; fat, kidney, muscle, liver, and brain. The levels of trans- residues were in the order; kidney, fat, liver, muscle and brain. The total radioactivity represented by the trans-isomer were higher than the cis-isomer in the liver, kidney, and brain.

Metabolism

Tashiro and Matsumura (1978) evaluated the metabolism of cis-chlordane and trans-chlordane *in vitro* by human and rat liver microsomes. Human and rat microsomes produced similar metabolites in similar proportions. In addition, the relative amounts of metabolites produced from the two isomers were not substantially different. The data, expressed as percent of the total starting material, are presented in Table 2.

Table 2. A Comparison of Metabolites of Cis-Chlordane and Trans-Clordane Produced *In Vitro* by Human and Rat Liver Microsomes (Tashiro and Matsumura, 1978).

Parent Material and Metabolites	Cis-Chlordane		Trans-Chlordane	
	Human	Rat	Human	Rat
1,2-Cl-chlordene	0.5[a]	2.1	0.4	-
Oxychlordane	1.0	5.2	1.2	2.3
cis-Chlordane	83.1	85.5	-	-
trans-Chlordane	-	-	85.2	86.4
1-exo-Hydroxy-2-Chlorochlordene	0.5	0.6	1.0	0.8
1-exo-Hydroxy-2-endo-chloro-2,3-exo-epoxychlordene	0.1	0.1	0.2	0.1
Human Metabolite	0.6	-	2.5	-
Chlordene chlorohydrin	5.7	3.8	4.5	7.9
Monohydroxylated dihydrochlordene	2.9	0.9	0.8	1.3
1,2-OH-chlordene	0.4	0.3	0.4	0.3
Trihydroxydihydrochlordene	0.6	0.2	0.5	0.0
1-exo-Hydroxydihydrochlordene	4.6	1.3	3.5	0.8

[a] Percent of total starting material.

The metabolism of trans-nonachlor by human and rat liver microsomes was substantially different. The human microsomes were essentially unable to metabolize trans-nonachlor. After a two hour incubation, 97.4% of the parent material was present while only 79.6% of the material in the rat microsomes was trans-nonachlor.

Tashiro and Matsumura (1977) concluded from an *in vivo* study in rats that the two endproducts of oxychlordane metabolism comprised a greater proportion of trans- metabolites present in feces than cis-metabolites (44% compared to 26.5%). However, this pathway is a significant one for both isomers. Mono- and dihydroxydihydrochlordene comprised 42% of the cis-derived metabolites in feces compared to 11.5% of the trans-derived metabolites. Brimfield and Street (1979) also found that the trans-isomer generated more oxychlordane than the cis-isomer *in vivo*.

Various metabolic pathways have been suggested based on both *in vitro* and *in vivo* studies (Barnett and Dorough, 1974; Brimfield et al., 1978; Tashiro and Matsumura, 1978; Balba and Saha, 1978; Poonawalla and Korte, 1971). The proposed schemes differ in the identity of metabolites based on *in vitro* or *in vivo* evidence, isomeric differences in the importance of metabolic pathways, and in the designation of terminal metabolites. Nomeir and Hajjar (1987) presented a synthesis of what is known about chlordane metabolism that proposes four metabolic pathways. These are shown in Figure 1.

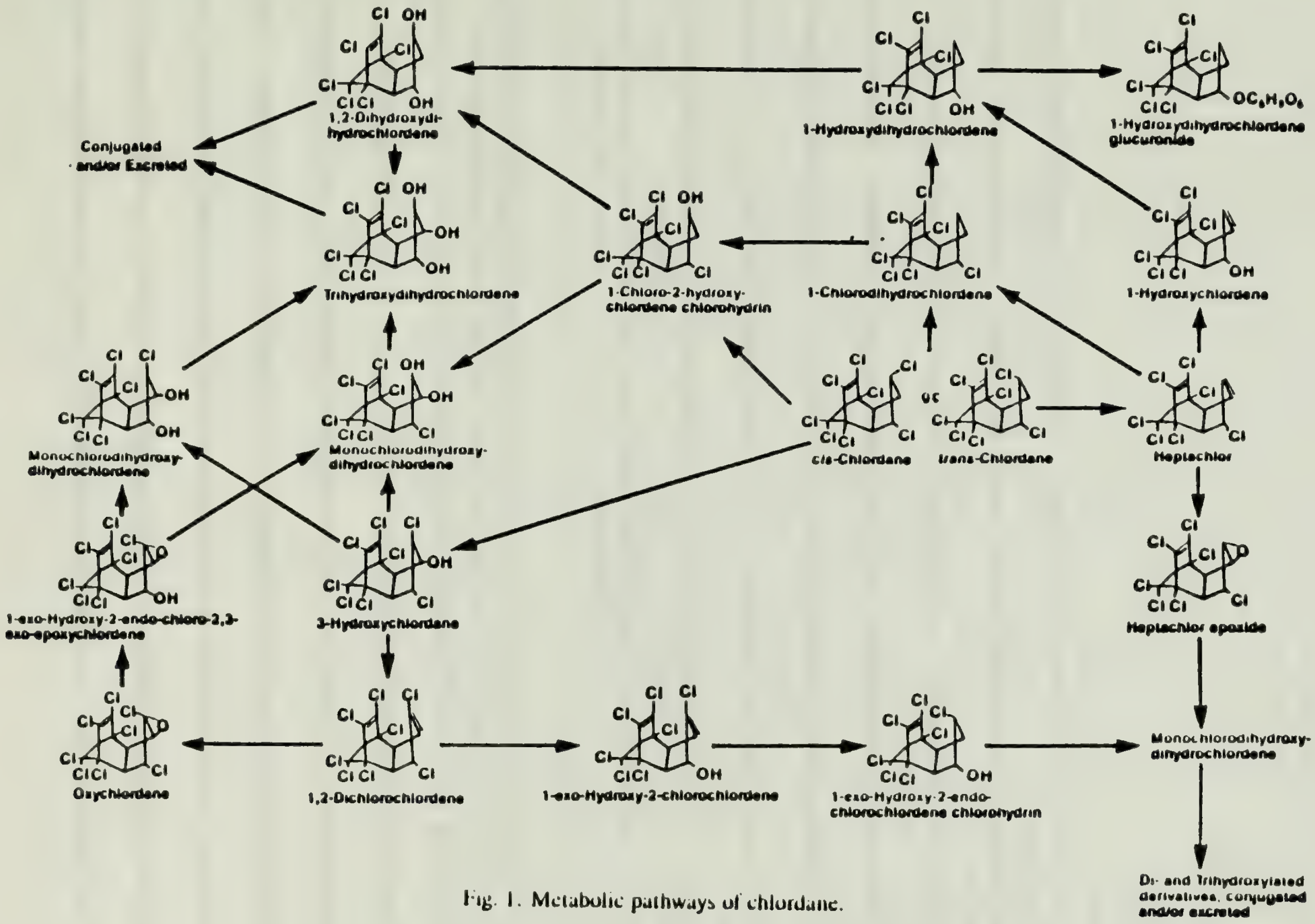
1,2-Dihydroxydihydrochlordene
Conjugated and/or Excreted
Trihydroxydihydrochlordene
1-Chloro-2-hydroxychlordene chlorohydrin
1-Hydroxydihydrochlordene
1-Hydroxydihydrochlordene glucuronide
$OC_6H_9O_6$
1-Chlorodihydrochlordene
1-Hydroxychlordene
Monochlorodihydroxydihydrochlordene
Monochlorodihydroxydihydrochlordene
cis-Chlordane
or
trans-Chlordane
Heptachlor
1-exo-Hydroxy-2-endo-chloro-2,3-exo-epoxychlordene
3-Hydroxychlordane
Heptachlor epoxide
Oxychlordane
1,2-Dichlorochlordene
1-exo-Hydroxy-2-chlorochlordene
1-exo-Hydroxy-2-endo-chlorochlordene chlorohydrin
Monochlorodihydroxydihydrochlordene
Di- and Trihydroxylated derivatives, conjugated and/or excreted

Fig. 1. Metabolic pathways of chlordane.

From: Nomeir and Haffar, 1987.

In one pathway, 3-hydroxychlordane is formed as a result of hydroxylation and is subsequently dehydrated to 1,2-dichlorochlordene finally leading to the formation of oxychlordane and 1-hydroxy-2-chlorochlordene. The intermediate can also undergo chlorine replacement by hydroxyl groups to form monochlorodihydroxylated and trihydroxylated derivatives. A second pathway is the formation of heptachlor through dehydrochlorination and then to 1-hydroxychlordene, heptachlor epoxide, or 1-chloro-2,3-dihydroxydihydrochlordene. Another pathway starts with dehalogenation of chlordane to form 1-chlorodihydrochlordene and subsequent conjugation. A final pathway involves the formation of 1-chloro-2-hydroxychlordene chlorohydrin and further metabolism to monochlorodihydroxy- and trihydroxy-derivatives of dihydroxychlordene.

Oxychlordane has been identified as a metabolite in rats (Tashiro and Matsumura, 1977; Brimfield et al., 1978, 1979; Polen et al., 1971), pigs (Schwemmer et al., 1970; Polen et al., 1971), rabbits (Balba and Saha, 1978), beagles (Polen et al., 1971), and cows (Polen et al., 1971) exposed to cis- or trans-chlordane.

Metabolites in Tissues

The metabolites identified in the blood, liver, and adipose tissue of male Wistar rats given a single oral dose of 100 ug/kg or 10 mg/kg trans-chlordane consisted primarily of oxychlordane (Ohno et al., 1986). The ratios of parent compound to total chlordane equivalents in the adipose tissue of rats dosed with 10 mg/kg were higher than the ratios for rats given 100 ug/kg at 8 hours, one, and seven days after dosing. The parent compound in rats in the 10 mg/kg group comprised 61%, 38%, and 26% of the total trans-chlordane equivalents while the proportion of total chlordane represented by parent compound in rats given 100 ug/kg rats was 11.5%, 18%, and 3.6% at the three time intervals. The ratio of parent compound to total chlordane residues in the liver was also greater for rats in the high dose group at eight hours (37% versus 6%). The relationship was reversed however at one and seven days. High dose rats had a ratio of 2.7% and 3.6% at one and seven days, respectively, while the proportion in low dose rats was 19.7% and 20.4%.

Oxychlordane was the major component of chlordane residues in the tissues of Sprague-Dawley rats given a single oral dose of 1 mg/kg HCS-3260 (Barrett and Dorough, 1974). Twenty-four hours after dosing, oxychlordane represented 53% and 63% of the radiocarbon in the adipose tissue of females and males, respectively. In rats dosed with 0.2 mg/kg cis- or trans-chlordane, oxychlordane comprised a greater percentage of the residues in the fat of animals given the trans-isomer. The metabolite accounted for 30 to 40% of the residues present in the liver and kidneys of HCS-3260 exposed rats. Water soluble and unextractable metabolites comprised 60% and 40% of the residues in liver and kidney, respectively. Negligible amounts of polar metabolites were found in adipose tissue. The cis- and trans-isomers were detected in adipose tissue, kidney, and liver. The relative amounts found in the tissues of males and females were not appreciably different. The administration of oxychlordane (0.2 mg/kg) resulted in the deposition of solely parent compound in adipose tissue.

The percent of ^{14}C-residues in fat, muscle, liver and kidney accounted for by oxychlordane increased as the length of treatment progressed from 14 to 56 days. After 56 days of treatment, oxychlordane represented 84 to 91% of the chlordane residues in the fat of rats fed 1, 5, or 25 ppm HCS-3260. Cis-and trans-chlordane comprised 7 to 11.5% and 1 to 4% of the total residues in fat, respectively. Oxychlordane comprised 48 to 53% of chlordane residues in the kidney, 55 to 66% in the liver, and 79% in muscle. Water soluble and unextracted metabolites accounted for 38 to 46% of the total in kidney, and 27 to 37% in liver at 56 days. Similar metabolites and proportions were present in male and female rats.

Twenty-eight days after treatment was suspended, oxychlordane comprised 85%, 91%, and 99% of the total residues in kidney, liver, and fat, respectively. The isomers were not detected, and water soluble and unextracted residues accounted for the remainder. The data at 56 days post-treatment were stated to be essentially the same.

More oxychlordane than parent compound was stored in the adipose tissue of male and female Holtzman rats fed pure cis- or trans-chlordane in the diet (50 - 200 ppm) for 11 to 15 days (Street and Blau, 1972). Less oxychlordane was stored in the adipose tissue of both sexes fed cis-chlordane compared to trans-chlordane. At 100 ppm in the diet, the ratio of the concentrations of oxychlordane to trans-isomer was 18 to 20 in females and 4.6 to 10 in males. The ratio of oxychlordane concentration to cis-isomer was 3.8 to 4.3 in females and 2.7 to 3.4 in males. Females stored a greater proportion of oxychlordane than males. The ratio of oxychlordane to parent compound in female rats fed 50 ppm technical chlordane was 6.7.

The ratio of oxychlordane concentration in fat to the dietary concentration of either isomer of a 50:50 mixture fed to white rats for one year was approximately one (Polen et al., 1971). Feeding of the cis-isomer at 15 or 45 ppm resulted in a storage ratio of 0.4 to 0.9, whereas 5 ppm resulted in a ratio of 1.6. The ratio for rats fed 75 to 150 ppm of the trans-isomer or 45 and 75 ppm of the mixture ranged between 1.0 to 1.4. The sample size used in this study was not stated and no statistical analysis of the data was conducted which makes any conclusions regarding the differences due to dose or isomeric composition tentative. The storage ratio in beagles fed technical chlordane for two years ranged between 0.8 to 1.2.

The pattern of oxychlordane storage in rabbits was similar to rats. Oxychlordane levels were higher than the parent compound in the tissues of four male rabbits given four doses of either the cis- or trans-isomer by capsule at four day intervals (Balba and Saha, 1978). On the fifth day after the fourth dose, the oxychlordane to chlordane ratio in fat was 4.6 for the cis-isomer and 10.1 for the trans-isomer. The difference between the isomers observed in adipose tissue was not replicated in other tissues.

Elimination

Elimination From Tissues

Chlordane levels in the fat of Sprague-Dawley rats fed 1, 5, or 25 ppm HCS-3260 for 56 days decreased by 60% during the first four weeks after dosing was terminated, but the rate of decrease slowed considerably during the subsequent four weeks (Barnett and Dorough, 1974). The authors reported that the dissipation of residues from tissues during 56 days on a normal diet was faster in males than females.

The elimination of chlordane residues from all tissues of male Wistar rats given a single oral dose of 50 ug/kg or 10 mg/kg trans-chlordane involved a rapid phase and a slow phase, except in adipose tissue (Ohno et al., 1986). The decrease in chlordane residues from adipose tissue showed only a slow elimination phase. The half-life (T1/2) of elimination for various tissues, calculated using a two compartment model and data collected over ten or twenty days, are shown in Table 3. The half-life for adipose tissue was calculated using a one compartment model and 20 days of elimination data. The dose level did not appear to change the rate of elimination from most tissues. However, from data collected over 20 days, elimination half-lives for skin and liver appear to be slower for lower dose levels.

Similar to blood clearance, elimination of chlordane residues from tissues was faster in male Sprague-Dawley rats given a single oral dose of 1 mg/kg cis-chlordane than in similarly dosed male C57BL/6JX mice (Ewing et al., 1985). The percent of peak concentrations in tissues was higher in mice at 12 hours after dosing except for adipose tissue. These differences were lessened considerably by 14 days, except in the testes and muscle.

Table 3. Elimination Half-lives for Trans-Chlordane in the Tissues of Rats Given a Single Oral Dose of 50 ug/kg or 10 mg/kg and Followed for 10 or 20 Days (Ohno et al., 1986).

	T1/2			
	50 ug/kg		10 mg/kg	
Tissue	T1/2[a]	T1/2[b]	T1/2[a]	T1/2[b]
	Hours	Days	Hours	Days
Blood				
(During 10 Days)	9.14	6.39	8.74	5.04
Brain				
(During 10 Days)	4.79	4.83	4.9	6.57
Muscle				
(During 10 Days)	6.52	8.87	5.16	7.34
Skin				
(During 10 Days)	12.98	7.13	11.84	5.48
(During 20 Days)		15.2		10.4
Kidney				
(During 10 Days)	8.17	4.83	9.18	4.42
Liver				
(During 10 Days)	9.62	5.05	5.86	4.33
(During 20 Days)		9.2		6.7
Adipose Tissue				
(During 20 Days)		9.1		8.4

[a] Half-life of rapid elimination phase
[b] Half-life of slow elimination phase

Excretion

The excretion of chlordane residues into feces and urine has been studied in rats, mice and rabbits. Excretion resulting from the continuous administration of the isomers alone or in a mixture, or in a single dose has been studied at various dose levels. The results in male and female rats and mice given a single oral dose are presented in Table 4 and 5.

Fecal excretion is the major route of chlordane elimination in rats and mice. At seven days after a single dose of cis-chlordane or HCS-3260, 86% or more of the administered dose was excreted in feces regardless of sex, or species. Trans-chlordane residues were excreted at a slower rate than cis-chlordane by male rats, but not by females. Excretion of trans-chlordane residues or those of a 3:1 cis:trans mixture seven days after a single dose was 1.4 to 2% of the administered dose in male Sprague-Dawley rats and was 5 - 6.2% in females. Urinary excretion was quite low in rats and mice but slightly greater in female rats than male rats. Urinary excretion of cis-chlordane residues in male mice (7.7 - 11% of administered dose) was comparable to that in female rats (8.5%).

Excretion in mice was biphasic. A small proportion of the sample exhibited a low rate of excretion, and a majority of the sample was capable of a high rate of excretion. The high excretors appeared to have a somewhat faster initial rate of fecal excretion of cis-chlordane residues than shown by male rats, although the percent of the administered dose excreted was similar between species at three days (Ewing et al., 1985). The size of the dose did not appear to have an impact on the rate of elimination in the rat. Seven days after a single 0.2 mg/kg dose of oxychlordane, only 18% and 3% of the administered dose was excreted in feces and urine, respectively.

Table 4. Excretion of Chlordane Residues in the Feces and Urine of Male Rats and Mice Given a Single Oral Dose of a Mixture of Chlordane Isomers, or the Cis- and Trans-isomer Delivered Alone.

		Percent of Administered Dose					
		Feces			Urine		
	Species, Material,	Days After Dosing			Days After Dosing		
Ref.[a]	Dose mg/kg	0.5	1	7	0.5	1	7
1	Sprague-Dawley Rat HCS-3260						
	0.2		> 60	88		1	2
	0.5			88			1.4
	1.0			94			2.7
2	Wistar Rats						
	trans-			65-78			0.8-1.2
	0.1		49			0.7	
	1.0		57			0.6	
	10.0		50			0.5	
3	Albino Rats						
	cis-						
	5.4		59	86		< 1	3
	trans-						
	9.7		27	66		< 2	6
4	Sprague-Dawley Rats						
	cis-						
	1.0		54.9	90.8		1.3	2.7
	C57BL/6JX Mice						
	cis-						
	1.0						
	Low	1.1	2.5	41.1	0	1.2	7.7
	High	34.4	69.3	86.8	3.3	7.3	11.0

[a] References

1. Barrett and Dorough, 1974
2. Ohno et al., 1986
3. Tashiro and Matsumura, 1977
4. Ewing et al., 1985

[b] A small proportion of the mice in this study had a low excretion rate.

Table 5. Excretion of Chlordane Residues in the Feces and Urine of Female Rats Given a Single Oral Dose of a Mixture of Chlordane Isomers, or the Cis- and Trans-isomer Delivered Alone.

		Percent of Administered Dose			
		Feces		Urine	
	Species, Material,	Days After Dosing		Days After Dosing	
Ref.	Dose mg/kg	1	7	1	7
1	Sprague-Dawley Rats HCS-3260				
	0.2	46.0	86.0	3.6	6.0
	0.5		91.0		6.2
	1.0		90.0		5.5
	Cis-				
	0.2	66.0	90.0	5.5	8.5
	Trans-				
	0.2	57.0	88.0	2.9	5.0

Reference
1. Barrett and Dorough, 1974

Ewing et al. (1985) suggested that high fecal excretion indicates that biliary excretion predominates in both rats and mice. Additional evidence was cited where two rats and two mice were injected intraperitoneally with 1 mg/kg cis-chlordane, and residues were measured in feces and urine after seven days. The proportion of the injected dose in the feces of rats and mice was 47% and 67%, respectively. The percent in the urine of rats and mice was 2% and 10%, respectively. The faster initial rate of excretion exhibited by orally treated mice was not evident in mice treated intraperitoneally. The authors proposed that chlordane is altered in the gut of mice with the absorption of metabolites in blood, further degradation, and subsequent excretion in bile. The ability to excrete chlordane residues into bile was demonstrated by Ohno et al. (1986) in Wistar strain rats dosed orally with 100 ug/kg trans-chlordane.

Female Sprague-Dawley rats fed 1, 5, or 25 ppm HCS-3260 in their diets for 56 days eliminated approximately 6% of the total consumed dose in their urine regardless of the dietary concentration. The amount of radioactivity excreted in feces increased as the dietary concentration increased. After 56 days of treatment, the proportion of the dose in feces was 70% in rats fed 1 ppm, 75% in rats fed 5 ppm, and 80% in rats fed 25 ppm. The authors suggested that absorption efficiency in the gut decreased with rising dose. Male rats fed 5 ppm HCS-3260 for 56 days excreted a greater proportion of the total dose in feces (80% versus 75%) and a smaller proportion in urine (2.3% versus 5.7%) than females. Rats were also fed the cis- or trans-isomer at 25 ppm in the diet for 14 days. A total of 75% of cis-chlordane residues were eliminated in feces compared to 65% of trans-chlordane residues. Five and three percent of the consumed dose of cis- and trans-derived residues were present in urine.

Two metabolic studies in rabbits given repeated doses of cis- or trans-chlordane for either 12 weeks (Poonawalla and Korte, 1971) or 17 days (Balba and Saha, 1978) found that 70% or more of the total dose administered was excreted. After 12 weeks in which rabbits were given a daily oral dose of 14.3 mg trans-chlordane, 70% of the dose was excreted daily. Twenty-two percent of the administered dose was found in feces, and 47% was found in urine (Poonawalla and Korte, 1971). Balba and Saha (1978) treated their sample for a shorter time period (1700 mg of material) and found that 48.5% and 46.1% of cis- or trans-chlordane was excreted in feces. Urine contained 28.4% and 35.8% of the administered dose.

SUMMARY

Chlordane residues have been identified in the blood of pesticide control operators, pesticide manufacturers, and the general population reflecting high occupational exposure and widespread environmental contamination. Chlordane concentrations in human tissues are related to the source, degree, and duration of exposure to chlordane.

Trans-nonachlor, a constituent of chlordane, and the chlordane metabolites, oxychlordane and heptachlor epoxide, have been measured in the blood of pesticide sprayers at average concentrations of 0.55 ppb, 0.29 ppb, and 0.29 ppb, respectively. Chlordane residues were not detectable in a nonexposed population (Saito et al., 1986). Although chlordane levels were not correlated with age or the duration of employment in a subset of pesticide sprayers with detectable chlordane blood concentrations, a high correlation ($p<0.001$) was found with the amount of chlordane sprayed ($r=0.68$) and the number of spraying days, particularly in the last three months ($r=0.81$). This subset of workers had a longer mean duration of employment (8.8 years versus 4.4 years) than another group with undetectable blood levels. The workers sprayed a greater amount of chlorane and on a more frequent basis.

Other evaluations of pesticide control operators have observed an association between blood levels of trans-nonachlor and oxychlordane with duration of employment (Takamiya, 1987). Oxychlordane and trans-nonachlor levels were correlated ($r=0.92$, $p<0.01$) suggesting a similar pattern of accumulation in humans for the two compounds.

Trans-nonachlor and oxychlordane were also the most prevalent components of the chlordane residues identified in the blood of 43 representatives of the

Japanese general population (Wariishi et al., 1986). Total chlordane residues in blood were 0.51 ppb and 0.46 ppb for males and females, respectively. Those people living in residences treated for termites within the past five years and those reporting frequent consumption of fish had significantly higher oxychlordane or trans-nonachlor concentrations than those who did not.

Chlordane residues have been quantified in human breast milk (Tojo et al., 1986; Miyazaki et al., 1980; Savage et al., 1981). Studies conducted in Japan have measured concentrations in breast milk in the following order; heptachlor epoxide > trans-nonachlor > oxychlordane > cis-nonachlor. Cis- and trans-chlordane were also identified at low concentrations in these samples. A study of chlordane residues in the breast milk of U.S. residents found that 72% of 378 samples collected in the Midwest contained detectable levels of oxychlordane. Mean oxychlordane concentrations in the total U.S. and Midwest samples were 95.8 and 80.6 ppb (fat adjusted), respectively. Mes et al. (1984) demonstrated that the transfer of chlordane residues in breast milk to nursing infants is potentially a significant source of exposure to the infants.

Little data are available concerning the absorption of chlordane in orally exposed animals. Ohno et al. (1986) estimated that 82.9% of an oral dose was absorbed by male Wistar strain rats during the first 48 hours based on a comparison of the pattern of accumulation and decline of trans-chlordane levels in venous blood after an oral or intravenous injection. Dose levels of 100 ug/kg or 1 mg/kg resulted in the calculation of similar absorption fractions.

The absorption of chlordane may be slower in mice compared to rats. The rise in blood concentrations in male Sprague-Dawley rats and male C57BL/6JX mice during two hours following a single oral dose of 1 mg/kg cis-chlordane was similar except that residues were higher in the rats.

Chlordane is distributed in animal tissues in a manner similar to other organochlorine chemicals. A single dose administered by gavage to Sprague-Dawley rats resulted in the detection of residues in several tissues including liver, kidney, brain and muscle, with the highest concentrations measured in adipose tissue (Barnett and Dorough, 1974). Concentrations in all sampled tissues increased in relation to dose level. Female rats stored higher levels in adipose tissue while males accumulated higher residue levels in the brain, muscle, liver and kidney when sampled at seven days after dosing. The pattern of distribution in tissues after the administration of the cis- or trans-isomer was similar to that exhibited by a 3:1 cis:trans mixture. However, trans-chlordane residues accumulated to a greater degree than ciso-chlordane. Other studies have described similar characteristics of chlordane distribution in male Wistar rats (Ohno et al., 1986), Sprague-Dawley rats, and C57BL/6JX mice (Ewing et al., 1985). Rabbits may accumulate a greater proportion of chlordane residues in the kidney (Balba and Saha, 1978).

Concentrations of oxychlordane, trans-nonachlor and heptachlor epoxide were higher in adipose tissue compared to the liver of an adult human male 58 days after an accidental ingestion of a chlordane solution (Olanoff et al., 1983). The fat to serum ratio of chlordane concentration in a 20 month old boy who drank technical chlordane was 147:1 eight days after ingestion when maximum fat concentrations were achieved and had increased by an order of magnitude after three months (Curley and Garrettson, 1969). The fat to blood concentration ratios in rats and mice 24 hours after dosing were much lower, 19.8 and 8.8, respectively.

Distribution in mice and rats given the same dose level differed in that maximum concentrations in muscle and testes were higher than the brain in mice, while the maximum levels in the brain of rats were higher than those in testes or muscle. Peak concentrations were attained in the tissues of rats and mice in two to four hours, and the peak concentrations were 1.5 to 3.3 times higher in rats for all tissues except muscle. Mice had higher blood chlordane levels than rats and cleared the residues from the blood at a slower rate.

The pattern of distribution in the tissues of rats is similar after a single dose or during a continuous treatment over 56 days. A steady state had not been reached in the fat of Sprague-Dawley rats fed 1, 5, or 25 ppm HCS-3260 at 56 days (Barnett and Dorough, 1974).

Various metabolic pathways have been suggested based on both *in vitro* and *in vivo* studies (Barnett and Dorough, 1974; Brimfield et al., 1978; Tashiro and Matsumura, 1978; Balba and Saha, 1978; Poonawalla and Korte, 1971). The proposed schemes differ in the identity of metabolites based on *in vitro* or *in vivo* evidence, isomeric difference in metabolic pathways, and in the designation of terminal metabolites. Nomeir and Hajjar (1987) presented a synthesis of what is known about chlordane metabolism that proposes four metabolic pathways..

Oxychlordane is a major metabolite in all species and has been identified in rats (Tashiro and Matsumura, 1977; Brimfield et al., 1978, 1979; Polen et al., 1971), pigs (Schwemmer et al., 1970; Polen et al., 1971), rabbits (Balba and Saha, 1978), beagles (Polen et al., 1971), cows (Polen et al., 1971) and humans (Savage et al., 1981) exposed to cis- or trans-chlordane. Trans-nonachlor is a major chlordane constituent found in human blood and tissues, but the compound has not been found in animals. An *in vitro* evaluation of cis- and trans-chlordane metabolism by human and rat liver microsomes found that the same metabolites were produced in similar proportions (Tashiro and Matsumura, 1978). The human microsomes were not able to transform trans-nonachlor while the rat microsomes had this capability. The rate of biotransformation may become slower as the chlordane dose increases (Ohno et al., 1986).

After 56 days of treatment, oxychlordane represented 84 to 91% of the chlordane residues in the fat of rats fed 1, 5, or 25 ppm HCS-3260 (Barnett and Dorough, 1974). Cis- and trans-chlordane comprised 7 to 11.5% and 1 to 4% of the total residues in fat, respectively. Oxychlordane also comprised 50% or more of the chlordane residues in the kidney, liver, and muscle. Similar metabolites and proportions were present in male and female rats. Less oxychlordane was stored in the adipose tissue of male and female Holtzman rats fed cis-chlordane for 11 to 15 days than when trans-chlordane was fed to the rats (Street and Blau, 1972).

The pattern of elimination of chlordane residues from tissues suggests a two compartment model for distribution (Ohno et al., 1986). A rapid and slow phase of elimination was measured in blood, brain, muscle, skin, kidney, and liver of male Wistar rats after a single 50 ug/kg or 10 mg/kg dose of trans-chlordane. Only a slow phase was measured in adipose tissue. This data indicates an elimination half-life for the rats of 8.4 to 9.1 days. Terminal elimination half-lives of 21, 34, and 88 days have been estimated for humans based on the decline in blood levels after an accidental ingestion of chlordane (Curley and Gerrettson, 1969; Olanoff et al., 1983; Aldrich and Holmes, 1969).

Fecal excretion is the major route of chlordane elimination in rats and mice. At seven days after a single dose of cis-chlordane or HCS-3260, 86% or more of the administered dose was excreted in feces regardless of sex, or species Barnett and Dorough, 1974; Ohno et al., 1986; Tashiro and Matsumura, 1977; Ewing et al., 1985). This also appears to be the case for human chlordane excretion (Aldrich and Holmes, 1969). Urinary excretion is quite low in rodents but is slightly greater in female rats than male rats (Barnett and Dorough, 1974). Urinary excretion of chlordane residues in mice was comparable to that in female rats. The size of the dose does not appear to have an impact on the rate of elimination in the rat. The excretion of oxychlordane is much slower. Seven days after a single 0.2 mg/kg dose of oxychlordane, only 18% and 3% of the administered dose was excreted in feces and urine, respectively (Barrett and Dorough, 1974). Long-term administration of chlordane to rats does not change the pattern of excretion observed in rats. Two studies of chlordane excretion in rabbits have reported conflicting results regarding the relative proportions of residues present in feces and urine (Poonawalla and Korte, 1971; Balba and Saha, 1978).

Biliary excretion of chlordane residues has been demonstrated in rats and mice (Ohno et al., 1986; Ewing et al., 1985). The slower absorption of chlordane into blood in mice, the higher maximum blood concentrations, and the lower tissue concentrations observed in mice compared to rats caused investigators to suggest that chlordane is altered in the gut of mice with the absorption of metabolites in blood, further degradation, and subsequent excretion in bile (Ewing et al., 1985).

Although only scant information is available concerning the disposition, metabolism, and elimination of chlordane in the mouse, the overall handling of chlordane by mice and rats appears to be similar. The data available for humans also indicate that the pattern of distribution, metabolism and elimination may be similar to rodents. Differences exhibited by humans included a lesser ability to metabolize and excrete trans-nonachlor, and a longer storage of chlordane residues in human tissues.

SYSTEMIC TOXICITY

CHLORDANE TOXICITY IN HUMANS

Acute exposure to chlordane via inhalation, or ingestion has resulted in gastrointestinal symptoms. Symptoms reported by case reports of accidental ingestion included upset stomach, nausea, abdominal cramps and diarrhea (U.S. EPA, 1980). Neurologic symptoms including headache, dizziness, vision problems, incoordination, irritability, excitability, confusion, incoordination, weakness, muscle tremors and convulsions have consistently been reported by case reports of accidental exposure to chlordane via inhalation, dermal absorption, or ingestion (U.S. EPA, 1980). Some poisoning incidents have resulted in death.

In 1976, a municipal water system was contaminated in Chattanooga, Tennessee as a result of a careless residential application of chlordane (Harrington et al., 1978). A total of 71 persons out of 105 residents in the 42 affected homes reported contact with the contaminated water through drinking, bathing, or cooking. Chlordane concentrations up to 1,200,000 ppb were detected. Thirteen individuals described symptoms compatible with chlordane exposure including gastrointestinal symptoms (nausea, vomiting, or abdominal pain) and neurological symptoms (dizziness, blurred vision, irritability, headache, paresthesia, or muscle dysfunction). All recovered within two days. Serum concentrations of transnonachlor and oxychlordane in symptomatic individuals were not significantly different from those reporting no symptoms. Serum oxychlordane levels were increased significantly after two months compared to the levels immediately after the incident in 11 out of the 13 people with symptoms. Mean levels in blacks were significantly higher than mean levels in whites.

Hepatic responses to acute exposure have not generally been described by case reports of chlordane intoxication. However, one report described liver toxicity in an infant following acute, prolonged dermal exposure to liquid chlordane (Balistreri et al., 1973). The child developed vomiting and seizures, jaundice, hepatomegaly, hypoprothrombinemia, and elevated transaminases. Serial liver biopsy revealed "marked universal centrilobular necrosis, fatty infiltration and minimal inflammation" five days post-exposure. Histology was reported to improve ten days after the incident, and after four months, hepatic architecture was intact. Serum transaminases were also normal after 10 days, although mild triglyceride accumulation persisted. Serum chlordane concentrations were not detected after the fifth day, and adipose concentrations were nondetectable after the tenth day post-exposure. Hepatocellular enzyme activity was increased in an adult male during the first week after he drank a 75% chlordane solution after his initial recovery from the acute phase of intoxication (Olanoff et al., 1983). Serum transaminases were nine times the upper limit of the normal range and then "gradually" returned to near normal values. The patient remained asymptomatic and did not develop jaundice. The elevated enzymes were attributed to chemical hepatitis. Jaundice was reported in some cases of inhalation exposure to chlordane associated with residential exposure after application for termite control (U.S. EPA, 1980).

Three studies of occupational exposure did not identify any toxic symptoms related to chlordane (Alvarez and Hyman, 1953; Princi and Spurbeck, 1951; Fishbein et al., 1964). However, these studies involved very small sample sizes, 15 to 35 workers, and did not evaluate the health status of terminated workers.

CHLORDANE TOXICITY IN EXPERIMENTAL ANIMALS

Mortality and Morbidity

High dietary levels of chlordane caused increased mortality, reduced growth, and the induction of tremors and convulsions in chronically treated rats. Osborne-Mendel rats, 20/sex/dose group were fed 0, 5, 10, 30, 150 and 300 ppm technical chlordane in their diets for 104 weeks (Ingle, 1952). Dietary concentrations of 150 and 300 ppm caused increased mortality that was significantly higher than controls at weeks 48 and 11, respectively. Mortality was highest among female rats. In dose groups fed 5, 10, and 30 ppm, no significant difference in mortality compared to controls was reported. Mortality among treated rats in the lower dose groups was 50 to 70% in males and 50 - 55% in females. Among controls, mortality was 60% in males and 45% in females. Weight gain was observed to be depressed in the two highest dose groups, however food consumption was not affected by chlordane treatment. Induced or spontaneous convulsions were observed in rats fed 150 or 300 ppm during the twelfth or twenty-sixth week, respectively. Tremors, but not convulsions, were induced in 30 ppm rats after 80 weeks in four females and two males. No tremors were observed in rats fed lower doses.

Significantly increased mortality was also reported in female Osborne-Mendel rats (50 rats/sex/dose) fed a high concentration of analytical grade chlordane in the diet for 80 weeks followed by an observation period of 29 weeks on a normal diet (NCI,1977). Mortality was not significantly increased in male rats. High dose male and female rats were fed a time-weighted-average of 407 and 241.5 ppm chlordane. Much higher concentrations had been included in the diet of these animals for the first 33 weeks of the experiment causing symptoms of toxicity and high mortality. The mean body weight of high dose males and females were consistently depressed compared to controls throughout the study, and high dose females had tremors at 44 weeks. Low dose rats were fed a TWA concentration of 203.5 ppm for males and 120 ppm for females. All treated groups had individuals with symptoms of toxicity including loss of body weight, rough and discolored hair, palpable masses, and tumors. These symptoms became progressively worse throughout the study.

No treatment-related effects on body weight and mortality were reported in F-344 rats, 80/sex/dose group, fed technical chlordane at dietary levels of 0, 1, 5, or 25 ppm for 130 weeks (RIASBT, 1983).

Survival was only 14% and 24% in male and female CD-1 mice fed 25 ppm technical chlordane in the diet for 18 months (IRDC, 1973). Survival was 51 - 73% in groups (100 rats/sex/dose group) fed 5 or 50 ppm chlordane and in controls. No effect on body weight gain or food consumption was reported.

A dose-related increase in mortality was observed in male B6C3F1 mice fed a TWA concentration of 29.9 and 56.2 ppm for 80 weeks followed by 10 weeks on a normal diet (NCI, 1977). Increased mortality was not observed in females fed 30.1 and 63.8 ppm chlordane (TWA concentration). Treatment had no effect on body weight gain in either sex. Tremors were reported in high dose males and females after 20 weeks. Alopecia was reported in male and female mice and a few individuals had a hunched appearance. Abdominal distension was also observed, predominantly in females.

Liver

Hepatic microsomal enzyme induction is caused by chlordane (Hart et al., 1963; Hart and Fouts, 1965) and may be one of the more sensitive biologic responses to chlordane exposure (Den Tonkelaar and Van Esch, 1974). Single doses of 10, 25 or 100 mg/kg body weight technical chlordane in male Holtzman rats (4 rats/group) significantly increased the metabolism of hexobarbital 8 to 22 days after an intraperitoneal injection (Hart et al., 1963). Aminopyrine metabolism was increased over controls after 8 to 29 days, and chlorpromazine metabolism was significantly increased by 25 mg/kg or 100 mg/kg on days 8 through 29 after injection. The lowest dose level used, 10 mg/kg, significantly increased the metabolism of hexobarbital and aminopyrine on the eighth day after chlordane administration. Doses of 10 mg/kg technical chlordane on three consecutive days increased hexobarbital, aminopyrine, or chlorpromazine metabolism on the first through twenty-second day after the last injection. Three consecutive doses of 10 mg/kg trans-chlordane increased the metabolism of the three drugs on days 1 through 22 or 29.

Three consecutive daily doses of 25 mg/kg trans-chlordane administered intraperitonealy to male Simonsen rats (5 animals/group) caused a significant increase ($p<0.02$) in liver weight, microsomal liver protein, and total microsomal protein in the liver (Hart and Fouts, 1965). Chlordane administration also increased the oxidation of NADPH by rat liver microsomes and increased the amount of carbon monoxide binding pigment. The stimulatory effect of chlordane on hepatic microsomal drug metabolism was suggested to occur through mechanisms similar to the action of phenobarbital. The authors noted that previous investigations using 25 mg/kg for three days in their laboratory had resulted in histological alterations in the hepatic cell similar to those caused by DDT and other related chlorinated insecticides and a proliferation of the smooth endoplasmic reticulum (SER) similar to that produced by phenobarbital.

Individual chlordane components and a metabolite, as well as the technical chlordane mixture induced hepatic enzymes when administered i.p. on days one and three of an experiment using immature male Wistar rats (4 rats/group). Technical chlordane, cis-chlordane, trans-chlordane, trans-nonachlor, and oxychlordane significantly increased the rate of DMAP N-demethylase formation and the rate of aldrin epoxidation *in vitro*, and increased cytochrome P450 content (p=0.01). Phenobarbital and methylcholanthrene also induced these hepatic enzymes. The percent liver weight was significantly increased by technical chlordane, and the two chlordane isomers. These materials, as well as oxychlordane, caused an increased amount of hepatic protein. Benz-a-pyrene hydroxylase was not increased by any chlordane material but was increased by phenobarbital (PB) and methylcholanthrene (MC). Ethoxyresorufin O-deethylase (EROD) was also not affected by chlordane administration but was increased by MC and a mixture of PB plus MC.

Den Tonkelaar and Van Esch (1974) evaluated microsomal liver activity in Wistar rats (6 rats/group) fed technical chlordane in the diet for two weeks. Expressed as percent of control enzyme activity, 10 ppm chlordane significantly increased the activities of aniline hydroxylase (AH) (134% of control level, p=0.025) and aminopyrine demethylase (ADPM) (239% of control level, p=0.025). The activity of hexobarbital oxidase (HexOx) was increased by 20 ppm chlordane (146% of control). Relative liver weights were increased only at higher dose levels. The authors established a no-effect-level (NOEL) for increased enzyme activity of 5 ppm (0.25 mg/kg/day) chlordane in the diet. The authors felt that two weeks was a sufficient time period to establish a NOEL for enzyme induction because several authors had reported that this effect already reaches a maximum after two weeks of feeding.

Liver microsomal enzyme activity and relative liver weights were increased in a dose-related manner in Osborne-Mendel rats administered 6.35 to 25 mg/kg/day chlordane (125 - 500 ppm) in the diet over 28 days (Casterline and Williams, 1971). Chlordane in the diet increased the activity of triacetinase in serum and liver, and aniline hydroxylase in liver. Nitroreductase activity in the liver was also increased. Brain cholinesterase activity was depressed in rats fed diets with 8% casein, and serum B-Glucuronidase activity was decreased by chlordane treatment. The effect of chlordane on enzyme activities at these dose levels was not different in males and females.

Velsicol Chemical Corporation conducted a chronic dietary study of technical chlordane fed to F-344 rats (80 rats/sex/dose group) at concentrations of 0, 1, 5, or 25 ppm for 130 weeks (RIASBT, 1983). No treatment-related effects were reported for body weight, mortality, hematology, clinical chemistry, or urinalysis endpoints. Increased liver weights were observed in males fed 5 and 25 ppm at 130 weeks (all survivors) but not at 26 or 52 weeks (8 rats/sex/group). A dietary concentration of 25 ppm was reported to cause increased liver weights and cellular swelling in females at weeks 26 and 52. Hepatocellular necrosis was reported at an incidence of 3/64, 13/64, 11/64, and 27/64 in male rats fed 0, 1, 5, and 25 ppm, respectively. The incidence was statistically significant in the 1 and 25 ppm groups. These data were subsequently reevaluated by a review board of pathologists who determined that no chlordane related hepatic effects were induced in the males. A dose level of 1 ppm (0.06 mg/kg/day) has been selected by the U.S. EPA as a no-observed-effect-level (NOEL) based on a finding of regional hepatocellular hypertrophy in in female rats fed 5 and 25 ppm (U.S. EPA, 1985, 1988 and 1989).

Investigators using computer image analysis of microscopic images of Feulgen-stained liver nuclei were able to make distinctions between the nuclei of chlordane exposed rats with CCl_4 induced liver cirrhosis, and chlordane exposed rats with healthy livers, and control rats (Mahon et al., 1979; Nair et al., 1980). The treatment of the diets of male Long-Evans BLULE rats with 0.1 mg/kg/day of 50:50 cis- and trans-chlordane for 20 weeks revealed decreased chromatin condensation, indicative of increased transcriptional activity, compared to controls. Nuclear area was also significantly greater in chlordane treated rats. DNA content was increased in rats with CCl_4 induced cirrotic livers and was highest in rats treated with both CCl_4 and chlordane. Further image scanning of nuclei with normal DNA content from control and chlordane treated rats found significant differences in chromatin condensation and distribution pattern related to chlordane exposure. The results of these studies indicate that chlordane caused nuclear changes in hepatic cells at a dose of 0.1 mg/kg/day after 20 weeks.

Osborne-Mendel rats (20 rats/sex/group) fed technical chlordane in the diet for 104 weeks had highly increased liver weights in the 150 and 300 ppm dose groups (Ingle, 1952). Liver sections taken from 5 males and females in each dose group showed marked effects at 150 and 300 ppm. Centrolobular hypertrophy was reported with a tendency toward cytoplasmic oxyphilia and hyalinization. Nuclear karyorrhexis, karylysis, and cell necrosis was observed limited to the central area. Fatty and albuminous degeneration in central and midzonal cords was evident, and varying degrees of bile duct proliferation was noted. Rats fed 30 ppm chlordane showed a slight degree of these effects while minimal changes were reported in rats fed 10 ppm. No hepatic changes were reported in rats fed 5 ppm chlordane.

ICR mice (80/sex/dose group) were fed 0, 1, 5, or 12.5 ppm technical chlordane for 104 weeks (RIASBT, 1983). Increased liver weights were reported for male mice fed 12.5 ppm at 52 weeks (8 mice/sex/group evaluated) and 104 weeks, and for females fed 12.5 ppm at 104 weeks. Hepatocellular swelling and degeneration was observed in males and females in the 5 (0.47 mg/kg/day) and 12.5 ppm groups. Liver necrosis was also significantly increased in males in the two dose groups.

An 18 month dietary study in CD-1 mice (100 mice/sex/group) observed increased liver weights and hepatocytomegaly at all dose levels used (0.65 to 6.5 mg/kg/day, 5 to 50 ppm in the diet) (IRDC, 1973, cited in Epstein, 1976). Mice treated by gavage for two weeks had increased liver weights at 8 mg/kg/day but not at four mg/kg/day (Johnson et al., 1986).

Kidney

Moderate to marked renal injury was observed in male and female Osborne-Mendel rats (20 rats/sex/group) fed 150, and 300 ppm technical chlordane in their diets for 104 weeks (Ingle, 1952). Kidney weight was increased and generalized congestion and hyperemia were observed with capillary congestion of glomerular tufts and albuminous materials in some of the capsular spaces. A moderate degree of capsular fibrosis was reported. Tubular necrosis and epithelial sloughing was also noted. Kidney damage was not reported in rats fed lower doses in this study or in rats or mice exposed to lower chronic doses of chlordane by other investigators.

Central Nervous System

Chlordane inhibited Na^+ -K^+ ATPase and mitochondrial Mg^{2+} ATPase activities in the brains of male Sprague-Dawley rats (Drummond et al., 1983). Rats, 12 per dose level, were fed diets containing 0, 25, 50, and 100 ppm chlordane for 12 weeks. Four rats per dose level were killed at 4, 8, and 12 weeks during the treatment period. A decreasing trend in body weight gain with increasing dose was observed, but it was not statistically significant. Rats in the highest dose group experienced slight convulsions. The decrease in ATPase activity was dose-dependent at each time period and was more pronounced in rats fed iron insufficient diets. At 12 weeks, ATPase activity decreased by 42% (Na^+ -K^+) and 52% (Mg^{2+}) in rats fed 100 ppm chlordane in iron sufficient diets and by 70% (both enzymes) in rats fed iron insufficient diets. The ATPase activity was significantly depressed in rats fed 25 ppm with iron sufficient diets at 12 weeks ($p<0.05$). Rats fed iron deficient diets had significantly decreased brain ATPase activities at 4, 8, and 12 weeks. A time-dependent decrease in Na^+ -K^+ ATPase activity was also reported that was statistically significant in rats fed iron deficient diets. The authors suggested that the reduction of enzyme activity may alter cellular processes such as active ion transport, neurotransmitter uptake and ATP synthesis.

The influence of chronic low-level administration of chlordane on mammalian cerebral electrical activity was studied by Hyde and Falkenberg (1976). Electroencephalogram activity was measured in Sprague-Dawley rats (4 rats/dose group) before, during, and after a 48 day exposure period in which daily i.p. injections of 0, 0.15, 1.75, and 25.0 mg/kg body weight technical chlordane were administered. Changes in neuroelectrical activity involving frequency, amplitude, and waveform were recorded. The alterations were induced by all treatment levels and were dose-related.

EEG readings from the left and right hemisphere recorded a decreased frequency of slow and medium waves with a concurrent shift toward faster wave frequency. Mean left hemisphere electrical activity in rats dosed 0.15, 1.75, and 25 mg/kg/day for 42 days increased by 56.4%, 63.2% and 80%, respectively, over pretreatment levels. These changes were significant compared to pretreatment levels and concurrently monitored control rats ($p<0.01$). The incidence of faster waves in the right hemisphere of rats in the three dose groups was increased by 44%, 52.6%, and 64.4%, respectively, compared to pretreatment levels. The increased incidence over pretreatment levels was highly significant in the two higher dose groups ($p<0.01$) and significant in the low dose group ($p<0.05$). The incidence in control rats also rose during the treatment period although to a lesser extent than in treated rats. This caused the difference between exposed and nonexposed to be not significant. Readings from contralateral leads in the left frontal and right occipital areas showed that electrical frequencies in the low to high dose groups increased by 47.1%, 52.2%, and 70.1%, respectively, compared to the pretreatment activity ($p<0.01$). The incidence of faster waves was also significantly greater than controls at 42 days ($p<0.01$).

Chlordane exposure also caused a significant change in the voltage of brain potentials. Amplitudes in the left hemisphere increased by 70.1% to 96.3% in treated rats over the pretreatment levels, and the bioelectrical activity was significantly different from control amplitudes in the two highest dose groups. Control levels also increased during the 42 day period.

Food deprivation for six days caused disturbances in the electrographs in all rats, but neuroelectrical excitability was particularly pronounced in chlordane treated animals. The mean frequency of discharges as well as wave voltage were significantly higher than controls.

The neurological changes continued during a 60 day period after chlordane treated was terminated. The mean frequency of discharges in the three treatment groups were 1.6 to 2.5 times higher than control values ($p<0.01$). Wave amplitudes also remained elevated and were statistically greater than control values at 60 days in rats treated with 25 mg/kg chlordane.

Alterations in wave morphology were observed in treated rats at all dose levels during the 48 day exposure period and throughout the 60 day recovery period. Similar waveforms were recorded only rarely in control rats.

The disturbances in electrocerebral activity were related to dosage and length of exposure. The authors concluded that the lack of recovery over 60 days indicated that persistent damage to the nervous system had occurred that was, perhaps, irreversible.

Immune System

The administration of trans-chlordane by gavage to adult female B6C3F1 mice for 14 days did not suppress cell-mediated or humoral immune response (Johnson et al., 1986). In contrast, *in vitro* studies of chlordane exposed mouse splenocytes demonstrated an ability to inhibit both types of immune response (Johnson et al., 1987).

Total lymphocytes were significantly increased in mice dosed with 8 mg/kg trans-chlordane for 14 days. A mean total of 7096 ± 524 lymphocytes/mm^3 were counted in these eight mice compared to 4678 ± 424 lymphocytes/mm^3 in 12 vehicle dosed controls. The elevation in mice (eight per group) dosed with 0.1, 1.0, or 4.0 mg/kg was not statistically significant. Two experiments designed to evaluate humoral immunity *in vivo* indicated that chlordane had no suppressive effect on this response. Sensitized mouse spleen cells from chlordane exposed mice had a number of IgM antibody-forming cells after challenge with sheep red blood cell (SRBC) that was similar to controls. The proliferative response of spleen cells from treated mice to the B cell-specific mitogen lipopolysaccharide (LPS) was also not significantly different from vehicle treated mice.

The proliferative response of spleen cells to the T cell mitogens Con A and PHA, and to allogeneic cells from female DBA/2 mice was investigated to determine chlordane related effects on cell-mediated immunity. A significant and dose-related increase in $[^3H]$thymidine uptake at several concentrations of Con A was demonstrated by the splenocytes of chlordane treated animals. The optimal concentration of Con A (5 ug/ml) produced a 38% and 50% increase in $[^3H]$thymidine incorporation over controls in mice dosed with 4 mg/kg and 8 mg/kg chlordane. A statistically increased proliferative response to PHA was also demonstrated in the two higher dose groups but only at the superoptimal mitogen concentration. An enhanced response of B6C3F1 mouse splenocytes to the addition of histoincompatible mouse DBA/2 cells *in vitro* (mixed lymphocyte response) was produced by chlordane exposure, significant at 8 mg/kg. On the other hand, an *in vivo* test of delayed hypersensitivity response to keyhole limpet hemocyanin indicated no difference between chlordane exposed and unexposed mice. Thus, an alteration in immunocompetence was not produced *in vivo* in chlordane exposed mice. A dose-related increase in liver weights was observed in treated mice which was significant at a dose of 8 mg/kg.

The suppression of both cell-mediated and humoral immune responses was produced in mouse spleen cells studied in several *in vitro* tests (Johnson et al., 1987). Micromolar concentrations of trans-chlordane suppressed the proliferative response of B6C3F1 mouse spleen cells tested for mixed lymphocyte response, B and T cell specific mitogen induced response, and antigen- (SRBC) specific immunoglobulin M (IgM) response.

Chlordane did not alter the time course of immune response over 5 days indicated by the day of maximum antibody forming cell response to SRBC by chlordane treated splenocytes. Maximum suppression of the antigen-specific response occurred when chlordane was added to the culture during the first two days. Treatment of SRBC cells with chlordane concentrations up to 100 uM did not alter their sensitizing function indicating that the immunosuppression was due to primary damage to splenocytes. Oxychlordane or chlordane incubated with a phenobarbital-induced liver S9 metabolizing system produced a reduced generation of antibody forming cells in response to SRBC that was similar to that observed with chlordane alone. The addition of human or mouse serum to cultured bone-marrow cells incubated with chlordane reversed the marked suppression of bone marrow cell proliferation by chlordane in serum-free cultures. The authors suggested that chlordane may bind to serum protein thus decreasing the amount of chemical distributed to immunocompetent organs.

SUMMARY

Acute exposure to chlordane via inhalation, or ingestion has resulted in gastrointestinal symptoms in humans. Symptoms reported by case reports of accidental ingestion included upset stomach, nausea, abdominal cramps and diarrhea (U.S. EPA, 1980). Neurologic symptoms including headache, dizziness, vision problems, incoordination, irritability, excitability, confusion, incoordination, weakness, muscle tremors and convulsions have consistently been reported by case reports of accidental exposure to chlordane via inhalation, dermal absorption, or ingestion (U.S. EPA, 1980). Some poisoning incidents have resulted in death.

The contamination of a municipal water system in Chattanooga, Tennessee in 1976 caused 13 out of 71 exposed residents to experience gastrointestinal and neurological symptoms similar to those reported in other case reports (Harrington et al., 1978).

Hepatic toxicity and liver enzyme induction resulting from poisoning incidents have been reported in an infant (Balistreri et al., 1973) and an adult (Olanoff et al., 1983). Jaundice was also reported in some cases of inhalation exposure to chlordane associated with residential exposure after application for termite control (U.S. EPA, 1980).

Three studies of occupational exposure did not identify any toxic symptoms related to chlordane (Alvarez and Hyman, 1953; Princi and Spurbeck, 1951; Fishbein et al., 1964). These studies involved very small sample sizes, 15 to 35 workers and did not evaluate the health status of terminated workers.

Female rats appear to be more sensitive to the toxic effects of chlordane on survival, growth, and neurological response. High dietary levels of chlordane, 150 ppm and above, caused increased mortality, reduced growth, and induced tremors and convulsions in chronically treated rats (Ingle, 1952; NCI, 1977). Dose levels of 30 ppm in the diet did not alter mortality or weight gain in rats (Ingle, 1952; RIASBT, 1983), although 30 ppm in the diet for 80 weeks caused tremors in male and female rats.

In contrast to the response observed in rats, male mice appear to be more sensitive to chlordane intoxication than females. A dose-related increase in mortality was not observed in CD-1 mice fed 5, 25, or 50 ppm technical chlordane in the diet for 18 months (IRDC, 1973). On the other hand, a dose-related increase in mortality was observed in male B6C3F1 mice fed a time-weighted-average (TWA) concentration of 29.9 or 56.2 ppm chlordane for 80 weeks (NCI, 1977). Mortality was not increased in females fed 30.1 or 63.8 ppm chlordane for 80 weeks. The high dose was associated with tremors in both sexes.

Hepatic microsomal enzyme induction has been demonstrated in rats by investigators using relatively high dose levels of technical chlordane (10 - 100 mg/kg body weight) (Hart et al., 1963; Hart and Fouts, 1965). Repeated dosing at lower dose levels resulted in effects that persisted for longer periods compared to single dose regimens. Enzyme induction was produced by cis- and trans-chlordane, trans-nonachlor, and oxychlordane as well as the technical mixture. The feeding of 10 ppm technical chlordane to Wistar rats for two weeks increased the hepatic activities of several enzymes, while a dose level of 5 ppm did not increase enzyme activities (Den Tonkelaar and Van Esch, 1974).

Increased liver weights were observed in male Fischer 344 rats fed 5 or 25 ppm technical chlordane for 130 weeks. Increased liver weight and hepatocellular swelling was reported in females fed 25 ppm for 130 weeks (RIASBT, 1983). A dose level of 1 ppm (0.06 mg/kg/day) has been selected by the U.S. EPA as a no-observed-effect-level (NOEL) based on regional hepatocellular hypertrophy induced in female rats (U.S. EPA, 1989).

Hepatocellular swelling and degeneration was observed in male and female ICR mice fed 0.47 mg/kg/day technical chlordane for 104 weeks (RIASBT, 1983). Liver necrosis was also evident in males, an indication of a greater sensitivity to chlordane toxicity on the part of male mice. Effects in the liver during chronic chlordane exposure have also been reported in CD-1 mice (IRDC, 1973).

Other systemic responses to chronic chlordane exposure have been demonstrated in rats including kidney damage (Ingle, 1952), decreased ATPase activity in the brain (Drummond et al., 1983), and disturbances in electrocerebral activity (Hyde and Falkenberg, 1976). Although evidence of cell-mediated and humoral immune suppression was supplied by *in vitro* studies (Johnson et al., 1986; Johnson et al., 1987), chlordane did not alter the immune response in an *in vivo* test of delayed hypersensitivity response.

The dose level of 0.06 mg/kg/day identified by the U.S. EPA as a NOEL may actually be associated with alterations in liver cells and other toxicological endpoints. Investigators using computer image analysis identified nuclear changes in the hepatic cells of male Long-Evans rats fed 0.1 mg/kg/day for 20 weeks with 50:50 cis- and trans-chlordane (Mahon et al., 1979; Nair et al., 1980). The authors concluded that the changes were evidence of enzyme induction. This may be defined to be an adverse effect because oxychlordane may be the active toxicological agent. Chlordane significantly increased neuroelectrical activity and altered brain wave morphology measured by electroencephalogram in Sprague-Dawley rats fed 0.15 mg/kg/day for 42 days.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Male

Very high doses of technical chlordane caused degenerative changes in the testicular tissue of sexually mature male Swiss mice (Balash et al., 1986). The mice, 10 per dose group, received 100 mg/kg or 300 mg/kg body weight by stomach tube for 30 days. The mean diameter of seminiferous tubules was decreased in the treated rats, and the smaller size was statistically significant in the highest dose group. Degenerative changes were reported in both dose groups among all stages of spermatogenic epithelium. The changes included atrophy, tubular edema, karyopyknosis, cytoplasmic vacuolation, and proliferation of Leydig cells. The changes were most pronounced in the 300 mg/kg dose group; 50% of the animals had died by day 21.

Virgin male Sprague-Dawley rats, 91 days of age, were fed 300 ppm technical chlordane for 90 days prior to sacrifice. Changes in ventral prostate androgen receptor content and peripheral testosterone metabolism were analyzed (Shain et al., 1977). The high dose level caused a significantly decreased mean ($0.02 > p > 0.01$) weight gain among treated rats but did not change the weights of the testes or ventral prostate compared to controls. The nuclear androgen receptor site content of the ventral prostate was profoundly increased in treated rats, 34,300 sites per cell compared to 7560 sites per cell in controls ($0.05 > p > 0.01$). Cytoplasmic receptor content was not altered. A decrease in protein, RNA, and DNA content of 13, 35, and 25% per gram of ventral prostate was observed. The changes in RNA content could not be attributed solely to cell loss since they were of lesser magnitude than the change in DNA content. The mean plasma testosterone concentration for pesticide-fed rats was not significantly different from controls. Therefore, the authors concluded that the alteration in ventral prostate androgen receptor homeostasis and protein, RNA, and DNA content were attributable to a primary effect on the ventral prostate.

Female

A very high dietary dose equivalent to 16 mg/kg/day chlordane (approximately 400 ppm) was reported to cause reduced fertility when male and female albino rats (seven pairs) were fed chlordane treated diets beginning at weaning of parental rats through the lactation of their offspring (Ambrose et al., 1953). Six pairs were fed the basic diet and served as controls. A decreased number of mated females delivered litters. The results were reported as the number of rats produced per total matings. Treated rats produced five litters out of 29 matings compared to 11 litters out of 12 matings in controls. The data were not evaluated statistically. No litters survived to weaning. In spite of the small number of mating pairs and the lack of statistical analysis, the data indicated that chlordane had an adverse effect on fertility. It is not possible to differentiate effects on reproductive capability from embryolethality since the number of pregnant females was not reported.

Keplinger et al. (1971) conducted a six generation study in Swiss white mice fed dietary concentrations of 25, 50, or 100 ppm chlordane. For each generation, 14 females and 4 males per dose group were bred at 120 days of age and were fed the chlordane treated diets through weaning of two litters. A detrimental effect on the fertility index (number of pregnancies as percent of the number of matings) was reported. The fertility index in mice fed 25 ppm chlordane in the sixth generation (first litter) and in mice fed 50 ppm in the second and fifth generations (first litter) was significantly lower than that in control mice. This effect on fertility did not occur in the second litters of any generation, were not consistently observed, and were not reported in mice fed 100 ppm. Therefore the significance of the reduced fertility is questionable.

The administration of chlordane (25 mg/kg i.p. once a week for three weeks) to mice significantly reduced the number of females that became pregnant (Welch et al., 1971). Females were mated after three weeks of treatment and were killed prior to the end of gestation. In three experiments, 3 - 20% of 34 - 36 treated females that were mated per test became pregnant compared to 27 - 51% of 24 - 35 control females per test. Litter size was not affected. When adult ovariectomized mice were injected i.p. with 25 mg/kg chlordane once a week for three weeks and then treated with estradiol-17B or estrone, a significant ($p<0.05$) reduction in the uterine wet weight response was observed in chlordane treated mice compared to controls. The amount of estrogen in the uteri of treated mice was also significantly reduced. Similar effects were also demonstrated in Sprague-Dawley rats. The administration of 2 - 5 mg/kg chlordane daily for seven days significantly reduced the uterotropic effect of tritiated estrone and decreased the amount of radioactivity in the uterus. The response was dose-related; daily doses of 1 to 50 mg/kg inhibited the uterotropic response by 15 - 75%.

Embryolethality/Fetolethality

Ova implantation was adversely affected in BALB/c mice given a single dose of chlordane isomers during pregnancy (Jang and Talamantes, 1977). An intraperitoneal dose of 50 or 100 mg/kg body weight cis or trans-chlordane was given to pregnant mice (12 - 17 per dose group) on day one of gestation. The number of implanted ova, indicated by the number of placenta in mice killed on days 11 - 14, was significantly reduced by the injection of 50 mg/kg cis-chlordane ($p<0.05$) but not by 100 mg/kg. The mean number of implantations in 13 pregnant mice dosed 50 mg/kg cis-chlordane was 8.08 compared to 10.35 implantations in the 20 vehicle-dosed controls. A reduced but statistically insignificant number of implantations was observed in mice dosed with 100 mg/kg trans-chlordane. The number of females that were pregnant upon examination was reduced in all dose groups. The reduction was most pronounced in high dose mice injected with either isomer and became significant in mice dosed with 100 mg/kg trans-chlordane. Forty-one percent of these mice were pregnant compared to 70% of controls ($p<0.05$).

Developmental Effects

Chernoff and Kavlock (1982) reported that 50 mg/kg chlordane administered by gavage to 25 pregnant CD-1 mice on days 8 through 12 of gestation had no effect on litter size or average weight of offspring during the first three days of life.

The feeding of 100 ppm chlordane to two generations of Swiss white mice significantly reduced pup viability during the first four days of life (Keplinger et al., 1971). A dietary concentration of 50 ppm significantly reduced pup viability in the offspring of the fourth and fifth generations.

Persistent effects on endocrine system function were reported in mouse offspring prenatally exposed to chlordane. Pregnant F2 hybrid mice were given a daily oral dose of 0.16 or 8 mg/kg body weight analytical grade chlordane in peanut butter throughout gestation to either the day of parturition or 22 days (Cranmer et al., 1978). Litters which had been culled randomly within treatment groups to four males and four females were evaluated at 101 days of age (15 per dose) when the mouse pituitary-adrenal axis is mature and fully functional. Resting plasma levels of corticosterone were increased by chlordane exposure in male mice ($p<0.01$) but not in female mice. Levels of the steroid in the plasma of males in the low dose group was 6.5 ± 1.2 ug/dl compared to 3.2 ± 0.6 or 2.6 ± 0.3 ug/dl in two control groups. Levels in high dose males were not significantly different from control levels. It should be noted that the mortality rate at seven days of age in the high dose group was 55%.

In vitro studies found no effect on adrenal corticosterone production or in the ability of the liver from the male mice to metabolize the steroid. Although corticosterone levels were not elevated in the plasma of females, a significant decrease in hepatic side-chain cleavage of the steroid was observed ($p<0.01$).

The effects on corticosterone control noted in adult offspring at 101 days of age persisted into middle age. Plasma corticosterone concentrations were also measured in dosed mice at 400 and 800 days of age (15 mice/age/dose). Plasma levels were significantly elevated in male mice at 400 days of age. Plasma corticosterone in males dosed 0.16 or 8 mg/kg/day *in utero* were 6.8 ± 1.3 and 8.1 ± 1.7 ug/dliter, respectively, compared to 4.5 ± 0.5 ug/dliter in controls ($p<0.05$). Plasma levels were also significantly increased in female mice but only in the low dose group (10.8 ± 1.4 ug/dliter versus 6.8 ± 0.8 ug/dliter, $p<0.5$). At 800 days, no differences from controls were observed in either sex. The authors suggested that the increase in plasma steroid concentration may be attributed to a decrease in the ability of the liver to metabolize corticosterone, or to the extended presence of the chemical in the tissues of the animals. The authors were not able to interpret the lack of a dose-response.

A depression in cell-mediated immunity (CMI) was induced in 101 day old, overtly normal offspring of BALB/c mice fed 0, 0.16, or 8.0 mg/kg chlordane daily throughout gestation (Spyker-Cranmer et al., 1982). The CMI response was measured by a contact hypersensitivity response to oxazolone in the ear (change in thickness) of sensitized mice (10 mice/sex/dose group) three days after a challenge. The depression in CMI was statistically significant in male and female mice prenatally exposed to 8.0 mg/kg chlordane. A maximum induration of 17.5 x 10^{-2} mm was observed at 48 hours in the ear of control male mice compared to 6.5 x 10^{-2} mm in 8 mg/kg offspring. Female controls had an induration of 17 x 10^{-2} mm while females from dams dosed with 8 mg/kg chlordane had an induration of 5 x 10^{-2} mm. The depression in CMI was verified in 100 day old BALB/c mice exposed *in utero* to 0, 4, or 16 mg/kg technical chlordane throughout a 19 day gestation period (Barnett et al., 1985b). A significantly suppressed delayed type hypersensitivity (DTH) reaction to oxazolone was reported in males and females exposed *in utero* to 4 mg/kg chlordane, and in females prenatally exposed to 16 mg/kg. Only two males in the higher dose group survived to 100 days of age.

At 30 days of age, the depression in the DTH response to oxazalone challenge was less pronounced and more variable (Barnett et al., 1985b). Chlordane exposed male and female mice had a maximum induration at 24 hours that was less than control mice but not significantly decreased. The authors suggested that the decline in immunocompetence over time indicated the accumulation of chlordane in the tissues of the offspring or, alternatively, damage to the regenerative capacity or maturation of lymphocyte stem cells.

In utero exposure to chlordane resulted in a dose-dependent enhanced survival to influenza type A virus infection in BALB/c mice (Menna et al., 1985). Survival in mock-treated control mice innoculated with a lethal dose of influenza A virus was 36% compared to 70%, 50%, 100%, and 100% in mice exposed *in utero* to 0.16, 2, 4, or 8 mg/kg technical chlordane. A dose-related increase in the level of serum antiviral antibody was observed in the primary response to virus inoculation in females exposed to 2, 4, and 8 mg/kg that was significantly greater than serum antibody levels in control females.

Barnett et al. (1985a) found that the influenza A virus-specific DTH response was significantly decreased in chlordane-treated offspring. A DTH response was elicited in mice infected with influenza A by injection of FLU-A antigen into the right hind footpad. Footpad thickness was significantly less in 38 day old offspring of dams fed 8 ($p<0.02$) or 16 mg/kg chlordane ($p<0.01$) compared to controls. T-cells harvested from chlordane-treated and vehicle-treated infected mice showed a similar ability to proliferate in response to influenza antigen in culture. Moreover, the titers of influenza virus in lung homogenates of offspring of treated mice were not significantly different from control mice infected with the virus on days 1, 3, 5, 7, and 11 postinfection. No differences were observed in the ability of the mice to resolve the infection. The authors suggested that chlordane enhances the number or activity of suppressor T cells which inhibit the generation of T_{DTH}-cells resulting in decreased immunopathology.

Prenatal exposure to chlordane (4 or 16 mg/kg) had no effect on splenic B cell or T cell populations in 30 day old offspring (Barnett et al., 1985b) or 100 day old offspring (Spyker-Cranmer et al., 1982) indicated by lymphocyte response in culture to varying concentrations of lipopolysaccharide (LPS) or phytohemagglutinin (PHA). Female offspring exposed to either 4 or 16 mg/kg chlordane *in utero* showed significant enhancement in their spleen cell Con A response when compared with vehicle-treated controls. Spleen cells from male offspring prenatally exposed to 4 mg/kg showed a small but significant increase in their response to Con A stimulation. According to the authors, since Con A affects a broad range of T-cell types, it is possible that chlordane enhanced the activity of one subpopulation of T-cell over others. In contrast to the enhanced response to Con A, the mixed lymphocyte reactivity, an *in vitro* assessment of an antigen-specific CMI reaction, was significantly depressed in male offspring in both dose groups. Although not statistically significant, female offspring of treated mice showed a dose-related increase in proliferation. The sex difference could not be explained, although the control response among females was quite low. The suppression of MLR in males was consistent with the depression of DTH.

No decrease in the primary T-cell dependent humoral immune response of offspring was observed in either 0.16 or 8 mg/kg chlordane dose groups at 100 days of age (Spyker-Cranmer et al., 1982). The number of IgM antibody producing spleen cells in male or female BALB/c mice immunized with SRBC was not significantly different between chlordane treated groups and controls.

Neonatal chlordane administration on days 2, 3, and 4 after parturition had an effect on endocrine development and body growth of female BALB/cCrl mice (Talamantes and Jang, 1977). Doses of 0.075 or 0.15 mg cis- or trans-chlordane were injected subcutaneously (2.5 or 5 mg/kg) and mice were followed for 12 weeks. The time of eye opening was significantly delayed in mice given the low dose of either isomer. The mean age of eye opening in mice receiving the cis- or trans-isomer was 14.52 ± .1 days and 14.43 ± 0.08 days compared to 14.05 ± 0.11 days in control mice ($p<0.01$). The mean age at vaginal opening was also delayed by all dose levels and chlordane isomers and was significantly greater in mice receiving 0.075 mg cis-chlordane (40.82 ± 0.42 days, $p<0.001$) or 0.15 mg trans-chlordane (36.5 ± 0.95 days, $p<0.05$) compared to controls (33.4 ± 0.9 days). Mice given the low or high dose of cis-chlordane had significantly lower mean body weights compared to control mice at week 4, 6, and 8 after treatment. Mice given the low dose of trans-chlordane had depressed body weights through week 10, while mice in the high dose group had lower mean body weights up to week 4. Mean body weights were comparable to controls at week 12. Lower mean pituitary weights ($p<0.02$) were observed in both dose groups receiving trans-chlordane at 106 days of age. Mice in the high dose group for both isomers exhibited ovary weights that were higher than controls.

Chlordane treatment has been shown to cause behavioral abnormalities in the offspring of albino mice treated during pregnancy (Al-Hachim and Al-Baker, 1973). Groups of six pregnant mice were given daily oral doses of 0, 1.0, or 2.5 mg/kg technical chlordane for seven days during the third trimester. Ten male and female mice per treatment group were selected from litters for behavioral evaluation. The acquisition of conditioned avoidance response was depressed in treated mice at 30 - 37 days of age. The mean number of responses in mice dosed 1.0 or 2.5 mg/kg and tested in 16 daily trials over a seven day period was 9.7 ± 2.9 and 9.7 ± 2.4, respectively, compared to 13.1 ± 2.6 response in control mice ($p<0.001$). Electroshock threshold (EST) was significantly elevated in treated mice in a dose-related manner ($p<0.001$). EST in mice dosed with 0, 1.0, or 2.5 mg/kg was 90.1 ± 27.8, 108.6 ± 19.7, and 134.9 ± 26.8 volts, respectively. The six week old progeny of mothers dosed with 2.5 mg/kg also were more active than the progeny of control dams. Offspring in the high dose group crossed more squares in an open field test during a daily three minute period for seven days than control offspring (137.7 ± 23.3 versus 93.9 ± 36.6). The activity of weanlings in the 1.0 mg/kg group was comparable to controls. The authors suggested that the neurological effects may be due to an inhibition of Na-K-ATPase activity in the brain.

SUMMARY AND WEIGHT OF EVIDENCE EVALUATION

High dose levels of chlordane have been reported to cause testicular damage in Swiss mice (Balash et al., 1986) and an increase in nuclear androgen receptor site content of the ventral prostate of Sprague-Dawley rats (Shain et al., 1977). Reduced fertility was reported in male and female rats fed 16 mg/kg/day chlordane from weaning of the parental rats through lactation of their offspring (Ambrose et al., 1953). Chlordane administered i.p. at a dose of 25 mg/kg once a week for three weeks reduced the number of mated Swiss Webster mice that were pregnant when killed prior to parturition (Welch et al., 1971). Chlordane caused a significant reduction in the uterotropic response to estrone or estradiol-17B and decreased the amount of steroid present in the uterus of adult ovariectomized mice injected with 25 mg/kg once a week for three weeks and in immature rats injected i.p. with a dose as low as 2 - 5 mg/kg daily for seven days. Chlordane is classified as a Probable Positive Reproductive II toxin based on the effects on fertility reported by Balash et al. (1986) in male mice, Ambrose et al. (1953) in albino rats, and Welch et al. (1971) in Swiss Webster mice. It is not possible to determine dose-response from these studies. The one multigeneration study which reported effects on fertility (Keplinger et al., 1971) did not observe a dose-related trend, and reductions in fertility were not consistently observed. The number of animals per dose level used in this study was relatively small, however. No studies reported a histopathological evaluation of female reproductive organs after chlordane exposure.

The administration of a single dose of cis- or trans-chlordane on day one of gestation to pregnant BALB/c mice resulted in a decrease in the number of females in all dose groups that were found to be pregnant upon examination on days 11 - 14 (Jang and Talamantes, 1977). The reduction was significant in mice dosed with 100 mg/kg trans-chlordane. Mice dosed with 50 mg/kg cis-chlordane had a reduction in the mean number of implantations recorded on gestation days 11 - 14. The lack of a dose-response makes it difficult to interpret the data on implantations.

While the administration of 50 mg/kg chlordane to pregnant CD-1 mice on gestation days 8 - 12 did not affect litter size, or the average weight of offspring during the first three days of life (Chernoff and Kavlock, 1982), a two generation feeding study using 100 ppm chlordane significantly reduced pup viability in one to four day old offspring of Swiss white mice (Keplinger et al., 1971). A dietary concentration of 50 ppm reduced pup viability in the fourth and fifth generations in a six generation test.

Persistent effects on endocrine system function were induced in mouse offspring exposed to chlordane *in utero* (Cranmer et al., 1978). Resting plasma levels of corticosterone were significantly increased in 101 day old male mice exposed during gestation to 0.16 mg/kg body weight analytical grade chlordane. Steroid levels were not affected in the female offspring dosed 0.16 or 8 mg/kg or in male offspring exposed to 8 mg/kg. Pup viability was reduced in the high dose groups, however. At 400 days of age, corticosterone levels were significantly elevated in males in both dose groups and in females in the lower dose group (Cranmer et al., 1984). This study indicates that plasma steroid levels were altered for a significant period of the animals' lives by exposure during the prenatal period to a relatively low maternal dose. The data provide additional evidence that the selection of 0.06 mg/kg/day as a NOAEL for systemic toxicity may not adequately protect the entire exposed human population.

Neonatal administration of 2.5 or 5 mg/kg body weight chlordane on days 2, 3, and 4 after parturition had an adverse effect on endocrine development and body growth of female BALB/cCrl mice (Talamantes and Jang, 1977). Mean age of eye opening and vaginal opening were significantly delayed, and 106 days old mice had lower mean pituitary weights and higher ovary weights than controls.

Although chlordane exposure did not cause a suppression in cell-mediated immunity in adult mice *in vivo*, a depressed contact hypersensitivity response to oxazolone in the ear of challenged, sensitized BALB/c mice was observed at 101 days of age in the offspring of mice exposed *in utero* to a maternal dose of 4, 8 or 16 mg/kg body weight chlordane (Spyker-Cranmer et al., 1982; Barnett et al., 1985b). A reduced, but nonsignificant hypersensitivity reaction was observed in similarly exposed 30 day old offspring. Survival from a lethal infection with influenza type A virus was enhanced by *in utero* exposure to a maternal dose of 0.16, 2, 4, or 8 mg/kg, and the influenza A virus-specific DTH response was significantly decreased in chlordane treated offspring (Menna et al., 1985; Barnett et al., 1985a). The authors suggested that chlordane enhances the number or activity of suppressor T cells which inhibit the generation of T_{DTH} cells resulting in decreased immunopathology. *In vitro* tests confirmed the ability of chlordane to suppress an antigen specific CMI reaction and indicated that the activity of one type of T cell population may be enhanced.

In utero exposure to maternal doses of 1 or 2.5 mg/kg technical chlordane during the third trimester resulted in a depressed acquisition of conditioned avoidance response in albino mice offspring at 30 - 37 days of age and increased the electroshock threshold in a dose-related manner (Al-Hachim and Al-Baker, 1973). These effects are consistent with the reduction in brain ATPase levels in chlordane treated rats reported by Drummond et al. (1983). Unfortunately, the neurobehavioral effects of chlordane have not been thoroughly investigated.

Chlordane is classified as a probable positive developmental toxin based on the reduction of pup viability in Swiss white mice at high doses in a multigeneration test (Keplinger et al., 1971). This classification is also based on the several tests involving exposure to lower doses during gestation which resulted in delayed effects in offspring. These effects included elevated plasma corticosterone levels in the offspring of F2 hybrid mice (Cranmer et al., 1978), the suppression of cell-mediated immunity in 100 day old BALB/c mice (Spyker-Cranmer et al., 1982; Barnett et al., 1985a and 1985b; Menna et al., 1985), and depressed acquisition of conditioned avoidance response and increased electroshock threshold in 30 day old albino mice (Al-Hachim and Al-Baker, 1973).

THE CARCINOGENICITY OF CHLORDANE

EPIDEMIOLOGIC EVIDENCE

Mortality in two occupational cohorts with chlordane exposure, pesticide applicators, and employees of a chlordane manufacturing plant has been followed by a few groups of investigators. An increased risk of lung cancer has been consistently suggested by these studies, but there are too many limitations to draw definitive conclusions. An possible risk of blood dyscrasias, including leukemia, has been indicated by case reports and two case-control studies.

Wang and MacMahon (1979) established a cohort of 16,126 male employees of three nationwide pest control companies with three months of employment or greater between 1967 and 1976. Since a majority of the total population was not eligible for inclusion in the cohort, a sample of 4000 was selected from a total of 27,957 excluded employees and evaluated. Eleven percent of this sample were female, 71% were employed for less than three months, and information was missing for 18%. Although information concerning race was not available for living individuals, 94% of the 269 workers for which death certificates were obtained were white and 5.6% were black.

A total of 331 deaths were identified by the Social Security Administration. Death certificates were not obtained for 42 individuals. The 42 deaths were included in the evaluation of cause-specific mortality by categorizing them according to the distribution observed in 269 death certificates. Cause and age-specific expected deaths were calculated from the U.S. mortality rates of white males and standard mortality ratios (SMRs) were estimated. The SMR for all causes of death was 84, perhaps due to the combination of an underascertainment of deaths and a healthy worker effect. Elevated SMRs of 115 and 173 were calculated for cancer of the lung and skin cancer which were not significant. Excess bladder cancer of marginal significance was observed with an SMR of 276 ($p<0.05$). Low SMRs were estimated for cancer of the digestive organs (46, 95% CI 22-95), diseases of the respiratory system (29, 95% CI 12-70), and diseases of the digestive system (55, 95% CI 31-98). When workers were categorized by occupation, SMRs remained elevated in the termite control operators group for skin cancer (148) and bladder cancer (245) remained, however the SMR for lung cancer was 87. The SMRs for cancer of the lung, skin, and bladder remained elevated in the other applicators category (131, 187, and 296). No trends in SMRs for any cause of death was observed when exposure within the cohort was classified by intensity. A cluster of excess lung cancer was observed among workers who were less than 45 years of age when employment began and were less than 50 years old when vital status was ascertained for the study. The increase was statistically significant. The authors reported that only 15% of the person-years were contributed by workers who had more than five years of employment and who were observed more than 10 years after first employment.

A second report of mortality in the cohort of pesticide applicators followed vital status through September 30, 1981 (MacMahon, Wang, and Monson, 1985). A total of 324 additional deaths were ascertained, and death certificates were obtained for 271 (84%). This increased the overall mortality in the cohort to 635 with death certificates obtained for 540 (85%). Again, the deaths of unknown cause were distributed according to the distribution of the deaths of known cause. The SMR for all causes of death was 82. The authors stated that because the SSA was used to identify deaths, actual deaths could be 10 - 15% higher than the number ascertained. The SMRs for cancer of the lung, skin, and bladder were still elevated, but the increase was not statistically significant (104, 150, 163, respectively). The elevated SMRs for lung and skin cancer were observed in the "other applicators" group (125 and 204), and only bladder cancer was elevated in termite control operators (227). No increasing lung cancer mortality trends were observed for duration of employment or for years since employment began using five year periods of 0-4, 5-9, and 10+ years.

The data from these reports do not lead to any conclusive determinations regarding the carcinogenicity of chlordane in pesticide applicators. If the cause-specific death distribution among the 95 deaths for which death certificates were not obtained were different from the group with death certificates, the resulting SMRs may actually be higher or lower than those calculated. The applicators were exposed to several kinds of pesticides including fumigants, botanicals, carbamates, chlorinated hydrocarbons and organophosphates. It is not possible, therefore, to assess the risks of chlordane exposure independently of other chemical exposures. In addition, other confounding factors such as cigarette smoking could not be evaluated. Finally, the study of cause-specific mortality among pest control operators is made extremely difficult by the high turn-over rate in employees.

An elevated mortality from lung cancer was reported for a cohort of pesticide applicators in Florida (Blair et al., 1983). Vital status as of January 1, 1977 was ascertained for 4411 pest control applicators licensed during 1965-1966. Although the minimum period of follow-up was only ten years, the SMR for lung cancer was 135 using U.S. age-specific mortality rates. The risk increased with the number of years licensed and was 101, 155, and 289 for those licensed for less than 10 years, for 10 to 19 years, and for 20 years or longer, respectively. The use of 1970 to 1975 Florida mortality rates did not alter the calculated SMRs.

No conclusions regarding the carcinogenicity of chlordane can be drawn from this study of pesticide applicators. Exposure to multiple chemicals were involved and no evaluations of smoking habits were conducted. However, the consistent finding of an elevated lung cancer mortality and its association with duration of exposure should be noted.

Three groups of investigators have assessed the mortality of workers at the only chlordane manufacturing plant in the United States employed beginning in 1946. Elevated, but nonsignificant SMRs for lung cancer were also observed in this chlordane exposed cohort.

The plant, located in Marshall, Illinois, began producing chlordane in 1946. This plant as well as employees at a heptachlor and endrin manufacturing plant in Memphis, Tennessee were studied by Wang and MacMahon (1979). All male workers employed for three months or longer prior to the spring of 1976 for whom sufficient identifying information was available were included in the study. A total of 570 workers (59%) were eligible at the Marshall plant and 835 workers (59%) were eligible at the Memphis plant according to this criteria. Missing information was responsible for the exclusion of 29% of the employees from the two plants. Vital status was ascertained from a Social Security Administration record search and from the results of an investigation by another researcher, Dr. Sidney Shindell, who had conducted a more thorough investigation of vital status in the same population. A follow-up period of 20 years or greater was attained for 35% of the study cohort at both plants (500 subjects). Follow-up periods of 10 to 19 years were achieved for 30.1%

(422 subjects) of the cohort, and less than 10 years for 34.2% (481 subjects) of the cohort. Expected cause-specific mortality rates were calculated using national mortality rates specific for sex, race and age and calendar time in five-year groups applied to the comparable person-years accumulated during the period of observation. Mortality could not be assessed by intensity of exposure because complete occupational histories were not available for all workers. Moreover, the serum pesticide levels of active workers in 1975 and 1976 did not correlate with the individuals' classification into exposure intensity categories determined by job category.

A total of 113 deaths occurred during the study period (1946 to 1976) at the two plants resulting in an SMR of 72 (95% CI 59-86). The SMR for lung cancer was elevated (134, 95% CI 73-228), but it was not significantly above that expected. The SMR for cerebrovascular disease (183, 95% CI 110-287) was significantly elevated. The SMRs for lung cancer and cerebrovascular disease for only the Marshall chlordane plant were also elevated (115 and 158). Although the authors stated that no relationship of lung cancer mortality with duration of follow-up was apparent, the SMRs for workers followed for less than 10 years and between 10 to 19 years were 200 and 240, respectively, indicating a rising trend. Only one lung cancer death had occurred with four expected among those followed for more than 20 years, however.

This study is inadequate to make a determination regarding the carcinogenicity of chlordane. The workers were exposed to multiple chemicals and the extent of chemical exposures to employees within the plants was not described. All employees, including office workers with little potential for extensive exposure were included, possibly resulting in an underestimate of risk. The size of the cohort was small and the number of deaths was small. It is possible that the SSA may not have identified all deaths in the cohort. Under-reporting deaths would also result in an underestimate of risk. Finally, confounding variables including smoking were not evaluated.

Ditraglia et al. (1981) included the Marshall chlordane plant in a larger study of mortality among workers employed at organochlorine pesticide manufacturing plants. Workers with at least six months employment prior to December 31, 1964 were selected into the cohort. Vital status was searched through the SSA, state motor vehicle offices, the U.S. Postal Mail Correction Service, and other federal and state agencies. Causes of death were coded by a nosologist from death certificates according to the ICDA in effect at the time of death. The cohort at the chlordane plant was comprised of 259 live subjects and 59 deceased. Nine individuals (3%) whose vital status was not known were assumed to be alive at the end of the follow-up period. The cohort represented 8,354 person-years of observation.

SMRs were calculated by using the U.S. white male age, calendar time, and cause-specific mortality rates. An excess of stomach cancer was reported, 3 observed compared to 0.99 expected (SMR = 303, 95% CI 61-885). SMRs were also elevated for rectal cancer (178), pancreatic cancer (110) and respiratory cancer (110). The increase was not statistically significant. An analysis of latency of all malignant neoplasms by the time since the date of first employment revealed no trend in the SMRs (66, 90, and 60 for 10 years or less, 10-19 years, and 20 years or more, respectively). Only 11 deaths from malignant neoplasms had occurred in the cohort by 1976, however. This, plus the small number of workers included in the study preclude any conclusions regarding the association of cancer with pesticide exposure. The degree of exposure within the cohort was not described and may have been quite variable.

The workers were exposed to other chemicals at the plant including chlorine and dicyclopentadiene. In addition, sex, race, and other confounders including smoking habit were not controlled in the study.

Shindell et al. (1981) reported a study of the 783 chlordane workers including those who had completed three months or more employment between January 1, 1946 and December 31, 1976. The cohort consisted of 689 white males, 10 non-white males, and 84 females. Vital status was ascertained on an individual basis for 97.4% of the cohort. Of 119 deceased, death certificates were obtained for all but five individuals. Mortality among the cohort was compared to U.S. race, sex, age, and cause specific mortality rates during the time period in which the cohort was at risk during and after employment. The cohort represented 6790 person-years of employment and 16,882 person-years of survival since the beginning of employment at the plant.

Overall mortality was lower among the workers than expected possibly reflecting a healthy worker effect and, along with deaths from cancer, showed no trend with increasing length of employment. No site-specific data was presented in this report.

A follow-up report of mortality in the cohort through June 30, 1985 was published in 1986 (Shindell and Ulrich, 1986). A total of 800 workers were involved; 706 white males, 7 black males, and 87 females, 161 of whom were deceased. The cohort represented 7,347 person-years of employment and 21,586 person-years of survival since the beginning of employment. Mortality from cancer was slightly, but not significantly, below that expected from U.S. cancer mortality. The SMR for production employees for all cancers combined was less than the SMR for nonproduction employees. Cancer of the lung was reported to account for about one third of the total cancers. The SMR for cancer of the lung was 86. In reply to a letter to the editor, Shindell presented the incidence of respiratory cancer and other cancers in relation to the number of years employed (Shindell, 1987). The SMRs calculated from observed and expected respiratory cancers indicates a rising trend with the number of years employed up to 20 years. These are shown in Table 6. Only four respiratory cancers were observed in individuals employed for more than 20 years.

Table 6. Observed and Expected Respiratory Cancers Among White Male Employees at the Marshall Chlordane Manufacturing Plant, 1946 to 1985 (Shindell, 1987).

Years Employed	Observed	Expected	SMR
0-4	3	6.1	49
5-9	1	1.3	77
10-14	2	1.5	133
15-19	2	1.2	166
20-24	2	1.6	125
25-29	1	1.7	59
30-34	1	1.0	100
35+	0	-	
	12	14.4	

The reports by Shindell and Ulrich (1986, 1987) are not adequate to determine the carcinogenicity of chlordane. The same problems described previously were present in this study.

Twenty-five previously unpublished case reports of blood dyscrasias following chlordane/heptachlor exposure were presented by Epstein and Ozonoff (1987) who also discussed the literature concerning pesticide associated blood disorders. The new reports brought the total number of case reports in the literature to 34. No potentially myelotoxic exposures besides chlordane/heptachlor were reported for 15 of the 25 unpublished cases. Exposure data was reported to be available for 16 of the cases of whom 75% involved homeowners and their families following home, lawn, and garden applications, particularly after home termite treatment. The other 25% involved professional applicators. Of the 23 classifiable dyscrasias, 14 were production defects, five were thrombocytopenic purpuras, and four were leukemias. Of the 27 previously published cases, 27 were production defects, one was thrombocytopenia, one was hemolytic anemia, and five were leukemias. The association of aplastic anemia with leukemia was discussed. Several conditions associated with aplastic anemia such as Down's and Faconi's syndromes, and ataxia-telangiectasia are also associated with the development of leukemia (Infante et al., 1978). Agents such as radiation, chloramphenicol, and benzene are associated with both aplastic anemia and leukemia. Infante et al. (1978) also reported five case reports of children with neuroblastoma who had prior exposure to chlordane formulations.

Epstein and Ozonoff (1987) discussed the difficulties of conducting epidemiological studies to evaluate the effect of pesticide exposure on the incidence of aplastic anemia. Aplastic anemia is a very rare occurrence. A six nation study of agranulocytosis incidence reported a population weighted average age and sex-standardized, region-specific agranulocytosis rate of 6.2 per million for 1980-1984. An adequate case-control study would require a very large prevalence of known domestic or occupational exposure to chlordane (25% to detect a doubling of risk with 113 cases). Wang and Grufferman (1981) conducted a case-control study of male residents of North Carolina who had died of aplastic anemia between the ages of 15 and 65 in the years, 1968 through 1977. Deaths were identified from State Department of Human Resources computer listings and 60 cases were selected after exclusion of those with malignancy. Occupations were determined from death certificates for the 60 subjects and 120 controls of the same age, sex, and race. Evaluation of the numbers employed in occupations involving pesticide exposure (farming and landscaping) revealed no dose-related association between pesticides and aplastic anemia. The relative risk was 0.67 (95% CI 0.26-1.7). Even so, the authors stated that "an association between the two cannot be totally ruled out in view of the several case reports of aplastic anemia following exposure to pesticides." They suggested that the "pesticide may induce idiosyncratic bone-marrow reactions in rare individuals."

In contrast to aplastic anemia, leukemia incidence in the United States is approximately 1 per ten thousand population per year (Epstein and Ozonoff, 1987). Two case-control studies have identified an elevated risk of leukemia in farmers in areas with a high production of corn for which chlordane was a registered pesticide. Blair and Thomas (1979) conducted a death certificate study of leukemia in Nebraska. A total of 1084 leukemia deaths were identified among white males, aged 30 and older from 1957 to 1974. Two controls were selected for each case matched for sex, race, county of usual residence, age at death, and calendar year of death. A higher risk for leukemia was identified among farmers with an odds ratio of 1.25 (95% CI 1.05 - 1.49). The risk was particularly high among farmers born after 1900 (OR=1.64) or less than 66 years of age at death (OR=1.83). The matched triplets were stratified according to demographic and agricultural characteristics of their county of usual residence. Elevated odds ratios were found for farmers from counties with high rates for leukemia mortality (OR=1.94), large corn acreage (OR=1.44), and high cattle inventories (OR=1.36). The level of corn production was the most persistent influence on the risk of leukemia among farmers. When the level of corn production was held constant, other agricultural factors did not significantly influence the risk.

An almost identical odds ratio was calculated for leukemia and Iowa farmers (OR=1.24, 95% CI 1.09 - 1.42) in a study of leukemia deaths between 1964 and 1978 which replicated the study design used by Blair and Thomas (1979) (Burmeister et al., 1982). Soybeans per acre (OR=1.5) and corn per acre (OR=1.6) were associated with increased leukemia mortality risks among farmers for those born from 1890 to 1900 but not in those born after 1900. Odds ratios were elevated for farmers born after 1900 for residents in counties with high use of herbicides and high number of egg-laying chickens.

EVIDENCE IN EXPERIMENTAL ANIMALS

The carcinogenic potential of chlordane has been evaluated in four chronic bioassays using mice and four chronic bioassays using rats.

Mice

Male C57BL/6N mice, a strain which does not spontaneously produce liver tumors, were fed 25 or 50 ppm concentrations of a 90% chlordane/10% heptachlor mixture in synthetic feed for a time period "in excess of one year" (Becker and Sell, 1979). All mice dying during the experiment were autopsied. A minimum of three animals per week were sacrificed and autopsied from each dose group starting six months after the beginning of the experiment. These included mice demonstrating two consecutive, progressive, significantly elevated AFP values, or a single value 10 times greater than normal. A total of 16 chlordane exposed mice, 27% of survivors, were found to have primary hepatocellular carcinoma. The first carcinoma was detected at 36 weeks. Multiple tumors, from one to seven per animal, were observed. Malignancy was demonstrated by the growth of some transplanted carcinomas in recipient mice. The incidence of hepatocellular carcinoma induced by chlordane exposure was greater than that induced by feeding 0.045 or 0.03% acetylaminofluorene (AAF). AAF induced hepatocellular carcinoma in 18% of survivors (seven mice) after 41 weeks of feeding. The 200 control mice, followed for a minimum of 18 months, demonstrated neither histological alterations of the liver or gross tumors.

Data regarding survival was not presented. The authors stated that the higher chlordane dose resulted in increased mortality that was "roughly proportional to concentration." It was also stated that the high dose mice survived in "reasonable numbers" when continuously exposed for longer than one year. The number of mice per dose group or the number of mice "at risk" was not stated, and tumor incidence was not presented in relation to dose level. Therefore, this study can not be used in a quantitative analysis of cancer risk. However, a 27% incidence of hepatocellular carcinoma in a strain with zero incidence in controls, is a finding pertinent to a qualitative evaluation of carcinogenic potential for chlordane.

The National Cancer Institute (1977) evaluated chlordane carcinogenicity in a 90 week bioassay using B6C3F1 mice. Analytical grade chlordane (65% cis, 35% trans) in time-weighted concentrations of 29.9 and 56.2 ppm were fed to male mice and 30.1 and 63.8 ppm were fed to female mice, beginning at 35 days of age and continuing for 80 weeks, followed by a 10 week observation period. The study design is presented in Table 7. The concentration of chlordane in feed was decreased due to toxicity after one week in low dose females and after 15 weeks in high dose females. Concentrations in the feed of males were increased after one week for the low dose group and after 15 weeks for the high dose group. Twenty matched controls per sex and dose group were followed, and a pooled group of 100 male and 80 female controls used in bioassays of dieldrin, chlordane, aldrin, heptachlor, diclorvos, and dimethoate was used for data analysis.

Survival in the low dose (p=0.02) and high dose male mice (p=0.01) was significantly depressed compared to controls (60 - 66%). Survival in females, 78 - 100%, was not altered by chlordane treatment. Body weight gain was not significantly different in treated mice compared to controls. Generalized tremors were observed in high dose males and females at week 20, and after week 50 alopecia and a hunched appearance were noted in a few mice. Abdominal distention was reported in all groups, but predominantly in females.

Over 90% of all animals were histologically examined. A very significant dose-related increasing trend in hepatocellular carcinoma occurred in both sexes. The cancer incidence is presented in Table 8. Incidence in males was significantly increased relative to incidence in controls in both the low and high dose groups. Incidence in high dose females was also significantly increased. The increased incidence remained significant after an adjustment using life-table analysis. Metastases to the lungs had occurred in two high dose males and three high dose females. The study design and reporting of this test in mice is adequate to conclude that chlordane is carcinogenic in B6C3F1 mice.

Table 7. National Cancer Institute Study Design for Chronic Bioassay of Chlordane in B6C3F1 Mice.

Sex and Treatment Group	Initial No. of Animals	Chlordane in Diet (ppm)	Time on Study		Time-Weighted Average Dose (ppm)
			Treated (weeks)	Untreated (weeks)	
			MALE		
Matched-Control	20	0	0	91	
Low-Dose	50	20	1		29.9
		30	79		
		0	0	10	
High-Dose	50	40	15		56.2
		60	65		
		0	0	10	
			FEMALE		
Matched-Control	20	0	0	91	
Low-Dose	50	40	1		30.1
		30	79		
		0	0	10	
High-Dose	50	80	15		63.8
		60	65		
		0	0	10	

Table 8. Heptocellular Carcinoma Incidence in A Chronic Bioassay of Chlordane Using Male and Female B6C3F1 Mice.

Dose Group	Hepatocellular Carcinoma # Mice/# Examined	Statistical Significance Fisher Exact Test	Statistical Significance After Life-Table Analysis
MALE			
Pooled-Control	17/92 (18%)	$< 0.0001^{a}$	$< 0.0001^{a}$
Low Dose 29.9 ppm	16/48 (33%)	0.04	0.001
High Dose 56.2 ppm	43/49 (88%)	< 0.0001	< 0.0001
FEMALE			
Pooled-Control	3/78 (4%)	$< 0.0001^{a}$	$< 0.0001^{a}$
Low Dose 30.1 ppm	4/47 (6%)	0.41	0.26
High Dose 63.8 ppm	34/49 (69%)	< 0.0001	< 0.0001

[a] Exact Test for Dose-related Trend.

A long-term bioassay conducted for the Velsicol Chemical Corporation (IRDC, 1973) induced hepatocellular carcinoma in CD-1 mice fed dietary concentrations of 0, 5, 25, or 50 ppm analytical grade chlordane for 18 months. A total of 100 male and 100 female six week old mice were begun on the test, and ten mice per sex per dose group were killed at six months. Histological evaluations were conducted on a range of organs from ten male and ten female mice from controls and the 50 ppm dose group at 18 months. Organs from other animals were examined only when their gross appearance was suggestive of neoplasia. The percent of mice histologically examined was 52%, 64%, 58%, and 55% for males, and 63%, 69%, 57%, and 57% for females in control, 5, 25, and 50 ppm dose groups, respectively. Body weight gain and food consumption were not affected, although survival was greatly decreased. A large number of animals were lost due to autolysis. Survival at 18 months was 14% and 24% in males and females fed 50 ppm, respectively, and was 51 to 73% in all other groups. Survival in male controls was also low (41%).

A dose-related increase in hepatocytomegaly was observed in treated mice. An increased incidence of hyperplastic liver nodules in males and females that was dose-related ($p<0.01$) in the two highest dose groups was reported by the investigators. An increased incidence (not significant) of hepatoma in males was reported in the 5 and 25 ppm dose group, but only four heptomas were identified in the 50 ppm group. The low incidence in the high dose group was probably a result of competing mortality.

The slides were re-examined by Dr. Reuber who diagnosed a highly significant ($p<1x10^{-9}$) incidence of hepatocellular carcinoma at 25 and 50 ppm (Epstein, 1976). His conclusions were confirmed by three separate pathologists. Hepatocellular carcinoma incidence (number of mice with tumors out of the number of mice examined) in males as determined by Reuber was 3/33 (9%), 5/55 (9%), 41/52 (79%), and 32/39 (82%) in the 0, 5, 25, and 50 dose groups, respectively. Incidence in females in the four dose groups was 0/45 (0%), 0/61 (0%), 32/50 (64%), and 26/37 (70%), respectively.

Another study conducted for Velsicol (RIASBT, 1983) fed 0, 1, 5, or 12.5 ppm technical grade chlordane to ICR mice (80/sex/dose group) for 24 months. These dose levels were reported to have no effect on survival or body weight gain. Groups of eight mice/sex/dose level were killed at 52 weeks, and those fed 12.5 ppm chlordane had significantly increased mean liver weights. Liver to body weight ratios were significantly increased in all dosed males at 52 weeks. Mean liver weight and liver to body weight ratios were significantly increased in males and females fed 12.5 ppm at 104 weeks. Liver to body weight ratios were also significantly increased in females fed 1 and 5 ppm at 104 weeks. A significant increase in the incidence of hepatocellular adenoma and hemangioma of the liver was observed in the 12.5 ppm males dying between 19 and 24 months or at the end of the study. Hepatic tumors were not increased in females. The data are presented in Table 9. The low incidence of hepatocellular carcinoma may reflect differences in strain susceptibility or differences in pathological criteria used to diagnose the tumors.

Table 9. Neoplastic Liver Lesions in ICR Mice Tested in a Chronic Chlordane Bioassay (RIASBT, 1983).

	Dose Groups (ppm)							
	MALES				FEMALES			
	0	1	5	12.5	0	1	5	12.5
Number Tissues Examined	71	71	72	72	72	72	71	72
Hepatocellular Adenoma	13	13	15	28*	1	1	3	1
Hepatocellular Adenoma - Carcinoma	3	3	7	9	0	0	0	1
Hemangioma	4	1	8	14*	0	2	1	0

* Statistically significantly different from control value ($p < 0.001$).

Rats

A long-term bioassay conducted for Velsicol Chemical Company using Fischer 344 rats found that chlordane induced a significantly increased incidence of liver adenoma and carcinoma when considered together (RIASBT, 1983). Eighty rats per sex per dose group were fed 0, 1, 5, or 25 ppm chlordane for 130 weeks. Groups of eight rats per sex and dose were killed for pathological analysis at 26 and 52 weeks. No dose-related effects on mortality, food consumption, water consumption, hematology, clinical chemistry, or urinalysis were reported. Liver weight was affected, and several toxic lesions were reported in the liver. Non-neoplastic liver effects reported by these investigators have been discussed in the section of this report reviewing systemic toxicity. A significant increase in adenomas was observed in the livers of males fed 25 ppm compared to control rats ($p<0.001$). No dose-related incidence of liver adenomas was reported in female rats. Of the 64 tissues from males examined in each dose group, hepatocellular adenoma was identified in 1, 1, 3, and 9 rats fed 0, 1, 5, and 25 ppm, respectively. Of the 64 tissues from females examined in each dose group, hepatocellular adenoma was identified in 0, 2, 0, and 0 rats fed 0, 1, 5, and 25 ppm, respectively. All tumors were identified after 104 weeks.

The U.S. EPA (1986) reported that the liver histopathology from the Velsicol bioassay was re-evaluated by Dr. Gary Williams who diagnosed three carcinomas which previously had been labelled adenomas and found three additional adenomas. Neoplastic liver lesions as diagnosed by Dr. Williams are presented in Table 10. Dr. Williams noted that only two liver slices were taken from rats without grossly visible tumors, perhaps resulting in an underestimation of liver tumor incidence in the rats. Dr. Williams concluded that the data provided weak evidence for chlordane related oncogenicity in the rats. The number of animals "at risk" of developing tumors was not presented by the review of this study (U.S. EPA, 1986) which would partially correct for other competing sources of mortality.

Table 10. Liver Neoplasms Identified by Dr. Gary Williams in a Re-evaluation of Data from a Long-Term Bioassay of Chlordane using Male Fischer 344 Rats (RIASBT, 1983) (64 tissues/sex/dose group examined).

	Dose (ppm)			
Neoplasm	0	1	4	25
Adenoma	1[a]	1	4	8[a]
Carcinoma	1[a]	0	0	2[a]
Total	2	1	4	10[b]

[a] One animal had both an adenoma and carcinoma.
[b] Significantly different from control value ($p < 0.05$).

The National Cancer Institute (1977) evaluated chlordane carcinogenicity in a 109 week bioassay using Osborne-Mendel rats. Analytical grade chlordane in time-weighted concentrations of 203.5 and 407.0 ppm were fed to male rats and 120.8 and 241.5 ppm were fed to female rats, beginning at 35 days of age and continuing for 80 weeks, followed by a 29 week observation period. The study design of this feeding study is presented in Table 11. The concentration of chlordane in feed was decreased due to toxicity after 33 weeks in low and high dose males and females and again after 14 weeks. Ten matched controls per sex and dose group were followed, and a pooled group of 60 male and 60 female controls used in bioassays of dieldrin, chlordane, heptachlor, diclorvos, and dimethoate was used for data analysis.

A significant dose-related trend ($p=0.003$) for increasing mortality was observed in high dose female rats. Mortality in control males was reported to be abnormally high (40%). Average body weight in high dose males and females was consistently lower than controls throughout the study, although the decrease was not statistically significant. Generalized tremors and weight loss were reported in high dose females at week 44. Tremors were not observed at any other time during the study, although adverse clinical signs including weight loss, rough and discolored hair, palpable masses, and multiple tumors were seen during the first year and increased in incidence during the second year.

Table 11. National Cancer Institute Study Design for Chronic Bioassay of Chlordane in Osborne-Mendel Rats.

			Time on Study		
Sex and Treatment Group	Initial No. of Animals	Chlordane in Diet (ppm)	Treated (weeks)	Untreated (weeks)	Time-Weighted Average Dose (ppm)
		MALE			
Matched-Control	10	0	0	109	
Low-Dose	50	400	33		203.5
		100	14		
		50	33		
		0	0	29	
High-Dose	50	800	33		407.0
		200	14		
		100	33		
		0	0	29	
		FEMALE			
Matched-Control	10	0	0	109	
Low-Dose	50	200	33		120.8
		100	14		
		50	33		
		0	0	29	
High-Dose	50	400	33		241.5
		200	14		
		100	33		
		0	0	29	

A variety of tumors were observed in the rats. Follicular cell adenoma and carcinoma incidence combined was significantly higher compared to pooled controls in high dose females (p=0.03 exact test for trend; p=0.01 after life-table adjustment). The number of rats with lesions compared to the number examined was 3 out of 58 (5%), 4 out of 43 (9%), and 6 out of 32 (19%) in the control, low and high dose groups, respectively. Incidence in males was 4 out of 51 (8%), 1 out of 34 (3%), and 6 out of 31 (19%) in the control, low and high dose groups, respectively. A significant dose-related trend was observed in males compared to the matched controls (p=0.02). A significant dose-related trend in malignant fibrous histiocytoma in male rats was also found relative to matched (p=0.014) or pooled controls (p=0.02). The pathologist commented that a large number of thyroid sections were not available for evaluation, and the spontaneous incidence of thyroid tumors was variable in control rats. It was concluded that the data was not sufficient to indicate a carcinogenic effect of chlordane for thyroid follicular cells or histiocytoma in the rats.

Hepatocellular carcinoma was identified in one treated rat, and no significant linear trend was observed when the carcinoma was combined with neoplastic nodules. However, incidence was significantly increased (p<0.05) in low dose females compared to pooled controls (23% versus 8%). The investigators concluded that chlordane was not carcinogenic in this rat strain.

No treatment-related neoplastic changes were observed in male and female albino rats (3 - 5 per dose level) fed 0, 10, 20, 40, 80, 160, 320, 640, or 1280 ppm technical grade chlordane for 400 days (Ambrose et al., 1953). Although mortality and growth retardation occurred in the higher dose groups, no effect on growth was reported for females fed 160 ppm or less or for males fed 10 or 80 ppm chlordane. The results of this study cannot be used to evaluate carcinogenicity because of the short treatment period and very small sample sizes.

Ingle (1952) fed Osborne-Mendel rats (20 rats/sex/dose group) dietary concentrations of 0, 5, 10, 30, 150, or 300 ppm technical grade chlordane for 104 weeks. Growth, food consumption, and survival were not affected in rats dosed with 30 ppm chlordane or less. No increase in neoplastic lesions were reported, however this study dose not represent an adequate investigation of the carcinogenic potential of chlordane. The number of animals per dose group was small, and most significantly, histopathologic sections were evaluated for only five males and five females per dose group.

MECHANISMS OF ACTION

Mutagenicity

The available literature on the mutagenic activity of chlordane was reviewed by the U.S. EPA Office of Pesticides and Toxic Substances, and a summary of the evaluation was published in the agency's *Carcinogenicity Assessment of Chlordane and Heptachlor/Heptachlor Epoxide* (1986). Nine out of 25 published articles representing 34 assays were judged to meet minimal data requirements of all genetic end-points necessary for regulatory purposes. These are the evaluation of gene mutation, chromosomal damage, and primary DNA repair. However, the available literature did not provide a "comprehensive assessment of genotoxic potential" for chlordane. The agency's conclusions are summarized in this section.

Gene Mutation

Five survey studies reported qualitative negative results in modified Ames assays using commercial or technical grade chlordane and either *Salmonella typhimurium* or *Escherichia coli* bacterial strains (Wildeman and Nazar, 1982; Probst et al., 1981; Gentile et al., 1982; Ashwood-Smith et al., 1972; Ficsor, 1972). Reverse mutation was not demonstrated in either the absence or presence of rat S9, plant S14 or corn 1S microsome activation systems.

Chlordane is classified as an inconclusive but presumptive positive mutagen because the chemical produced a qualitative (primary data not reported) positive result in a survey study evaluating reverse mutation at the waxy locus in *Zea mays* (Gentile et al., 1982). Chlordane is also classified as presumptively genotoxic in yeast. Commercial chlordane in the presence of rat S9 or corn 1S microsomes and technical chlordane in the presence of rat S9 microsomes produced positive results in a *Saccharomyces cerevisiae* D4 mitotic gene conversion assay (Gentile et al., 1982).

Two studies considered acceptable by the U.S. EPA found that technical or trans-chlordane did not produce histidine prototrophy in *S. typhimurium* in the dose ranges tested in nonactivated systems (0.1 to 10 ug/plate) or using rat or hamster S9 cells (10 to 1000 ug/plate or 100 to 10,000 ug/plate depending on the bacterial strain used) (Haworth et al., 1986; Mortelmans et al., 1986).

Chlordane, when tested for gene mutation in mammalian cells, has produced evidence of mutagenicity in some tests but not in others. Chlordane was classified as an unconfirmed presumptive mutagen in Chinese hamster lung cells. A dose level which resulted in 44.4% cell survival (10 uM) induced a fifteenfold increase in ouabain-resistant mutants in 30 replicate cultures

(Ahmed et al., 1977). However, the results were inconclusive because only a single dose was used, no positive control was tested, and two compounds considered to be only weak mutagens, carbaryl and 2,4-D, exhibited strong mutagenic activity in this test. In another assay using a Chinese hamster lung cell line (V79) that was not included in the U.S. EPA review, technical chlordane did not increase the number of 6-TG or diphtheria toxin (DT) resistant cells when cultured in the presence of the chemical for two days (Tsushimoto et al., 1983). The test was not conducted in the presence of metabolic activation however, and only one dose level was tested (1.6 ug/ml) which resulted in only 13.6% or 0.57% cell survival.

A National Toxicology Program study reported that chlordane, only in the absence of metabolic activation, induced forward mutation at the TK locus of L5178Y mouse cells (NTP, 1985). Technical chlordane did not increase the frequency of 6-thioguanine resistant (6-TG^r) mutants in primary adult rat liver epithelial cells (ARL) (10^{-3} to 10^{-6} M) or human foreskin fibroblast (D-550) (10^{-4} M in the presence and absence of rat hepatocytes) up to cytotoxic doses (Telang et al., 1982; Tong et al., 1981).

Chromosomal Aberrations

Technical chlordane or a 3:1 cis:trans chlordane mixture did not induce a dominant lethal response when administered in a single 50 or 100 mg/kg dose by i.p. or gavage to male mice sequentially mated for six weeks (Arnold et al., 1977). A dominant lethal effect was not produced in mouse germinal cells by technical chlordane or the cis- or trans-isomers when administered in a single 48 or 240 mg/kg i.p. dose or at 50 mg/kg/day by gavage for five consecutive days (Epstein et al., 1972). Animals given 240 mg/kg i.p. or 50 mg/kg/day by gavage experienced some mortality, but all surviving males were sequentially mated for eight weeks and no significant differences in pregnancy, total implants, and early fetal deaths were reported. Cis-chlordane administered 42, 58, 290, or 210 mg/kg i.p. or 75 mg/kg/day for five days, produced no response in a dominant lethal assay (Epstein et al., 1972). Trans-chlordane also did not produce chromosomal aberrations in Chinese hamster ovary cells *in vitro* when tested at cytotoxic doses and less (NTP, 1985).

DNA Repair

Two assays for gene conversion in *Saccharomyces cerevisiae* strain D4 were concluded to be presumptively positive, but inconclusive (Gentile et al., 1982; Chambers and Dutta, 1976). Unscheduled DNA synthesis was not induced by the highest noncytotoxic dose (10^{-5} or 100 nmol/mL) of chlordane in rat, mouse, and hamster primary hepatocytes (Maslansky and Williams, 1981; Probst et al., 1981).

A UDS assay conducted in SV-40 transformed human fibroblasts (VA-4) with nonactivated and rat S9-activated doses of chlordane of 1, 10, 100, and 1000 uM, was reported to cause a significant increase in UDS over the control at all doses (Ahmed et al., 1977). The study was concluded to be inconclusive by the U.S. EPA because primary data was presented for the lowest dose only and dose-response relationships could not be assessed. Other limitations to the study were the use of a virus transformed cell line, the possibility that scheduled DNA synthesis may not have been completely blocked causing a false positive result, a lack of confirmation by replication, and the observation of strong responses by substances considered to be only weakly mutagenic in this assay. Chlordane was classified as a presumptive but unconfirmed genotoxic for SV-40 transformed human cells.

A less than two-fold increase in sister chromatid exchange (SCE) was produced in human hymphoid LAZ-007 cells exposed to nonactivated and rat S9-activated chlordane (10^{-5} to 10^{-6} M) (Sobti et al., 1983) but the results did not satisfy U.S. EPA criteria for a positive response.

A dose-related and highly significant increase in the frequency of SCE was observed in the intestinal tissue of mudminnows exposed *in vivo* to four doses of chlordane (5.4×10^{-9} to 5.4×10^{-12} M) for 11 days (Vigfusson et al., 1983).

Trans-chlordane induced SCE in an *in vitro* test using Chinese hamster ovary cells in the absence of metabolic activation (NTP, 1985).

DNA Binding

Radioactively labelled cis-chlordane was reported to bind irreversibly with DNA added to cofactor-fortified hepatic microsomes from male C57BL/6J mice (Brimfield and Street, 1981). Both the cis- and trans-isomers bound to endogenous protein and RNA. Induced microsomes increased binding to protein and DNA. The inhibition of epoxide hydratase activity reduced cis-chlordane related binding to DNA indicating that the DNA-cis-chlordane adduct is a product of epoxide cleavage. The authors stated that oxychlordane is relatively inert to epoxide cleavage and therefore may not be involved in this mechanism.

Epigenetic Activity

Chlordane caused an increased incidence of liver neoplasms in male B6C3F1 mice after the administration of diethylnitrosamine (DEN) that was significantly greater than the incidence induced by DEN alone (Williams and Numoto, 1984).

The mice, beginning at eight weeks of age, were fed 20 ppm DEN for 14 weeks, followed by a four week recovery period and then the administration of 25 or 50 ppm chlordane in feed for 25 weeks. At the end of the 43 week study, survival was decreased in all groups receiving DEN. A decrease in weight gain was caused by 50 ppm chlordane that was greater than 10% compared to controls.

Chlordane administration caused an increase in the number and area of glucose-6-phosphatase (G-6-P) deficient foci in the mice livers compared to DEN alone. The number of foci /cm^2 in mice given DEN alone, or DEN plus 25 or 50 ppm chlordane were 1.27 ± 1.07, 3.01 ± 1.28, 3.83 ± 2.07, respectively. The number was significantly increased by both chlordane dose levels ($p<0.01$). The area of foci in the livers of mice fed 50 ppm chlordane was significantly treated than the area of foci in mice fed DEN alone. The administration of chlordane without DEN in the diets of mice for 25 weeks did not increase the number of liver foci.

The incidence of liver adenomas and carcinomas in untreated controls, mice fed DEN alone, DEN plus 25 ppm chlordane, or DEN plus 50 ppm chlordane was 10.7%, 40%, 81% and 81.8%, respectively. The enhanced incidence in chlordane treated mice was significant ($p<0.01$). The promoting activity of chlordane was greater than that caused by DDT, used as a positive control, which produced an incidence of 66.7%. The multiplicity of neoplasms per liver was increased by chlordane as well. Mice fed DEN plus either 25 or 50 ppm chlordane had 2.05 ± 1.68 ($p<0.005$) and 2.36 ± 1.94 ($p<0.005$) liver neoplasms, respectively compared to 0.65 ± 1.01 neoplasms in the liver of mice fed only DEN for 14 weeks. The incidence of liver tumors produced by 50 ppm chlordane without DEN was comparable to the incidence in untreated controls (12%). The administration of 50 ppm chlordane prior to treatment with DEN did not result in a significant enhancement of liver tumor incidence as 23.1% of the mice developed neoplasms. Interestingly, the feeding of N-2-fluorenylacetamide (FAA), DNA-reactive liver carcinogen, at 200 ppm prior to DEN also did not induce an effect comparable to the administration of DEN followed by FAA (14.3% versus 70.6%). Although mice exposed to DEN developed squamous cell papillomas and carcinomas in the forestomach and pulmonary adenomas, the incidence of these neoplasms was not enhanced by chlordane.

Chlordane demonstrated an ability to inhibit intercellular communication between male Fischer F344 rat liver cells in culture (Telang et al., 1982). The inhibition of intercellular communication was indicated by the survival of TG^r HGPRT$^-$ cells when cultivated with TG^s HGPRT$^+$ cells. Chlordane was most effective at a dose of 5 x 10^{-6} M cultivated with the hepatic primary cells for 2 hours. This dose was associated with a 53.9% colony forming cell efficiency compared to 57.1% in cells exposed to the solvent DMSO or 62.6% in untreated cells. Benzo[a]pyrene, a DNA-reactive carcinogen, did not inhibit metabolic cooperation at noncytotoxic concentrations in this assay.

Technical chlordane did not decrease metabolic cooperation at noncytotoxic doses in the same assay using Chinese hamster V79 cells (Tsushimoto et al., 1983). The cells were cultured with chlordane or 12-O-tetradecanoylphorbol-13-acetate (TPA), the positive control, and 6-thioguanine for tree days. After removal of the chemicals, the cultures were grown in selective medium for four more days. At a concentration of 0.41 ug/ml the recovery of 6-TG^r cells was 31% comparable to that in DMSO solvent treated controls (30%). The recovery in cells treated with DDT or TPA was significantly increased compared to solvent treated controls (48% versus 38% and 67% versus 30%, respectively).

Metabolic activation may have been required for the inhibitory capacity of chlordane to be realized.

SUMMARY AND WEIGHT OF EVIDENCE FOR CARCINOGENICITY

Cancer mortality has been evaluated in pesticide applicators and chlordane manufacturing workers, and although statistically not significant, a consistent elevation in the standard mortality ratio (SMR) for lung cancer has been reported. Increased SMRs for lung, skin, and bladder cancer were reported in pesticide applicators (Wang and MacMahon, 1979; MacMahon, Wang, and Monson, 1985). A statistically significant cluster of excess lung cancer was observed among workers who were less than 45 years of age when employment began and were less than 50 years old when vital status was ascertained for the study. SMRs were not related to the duration of employment, however.

Blair et al. (1983) reported an elevated, nonsignificant SMR for lung cancer in Florida structural pest control workers which demonstrated a relationship to the number of years of licensure. Elevated, but nonsignificant SMRs for lung cancer were also observed in employees at a U.S. chlordane manufacturing plant (Wang and MacMahon, 1979; Ditraglia et al., 1981). Data presented by Wang and MacMahon (1979) and Shindell (1987) showed a rise in the SMR with increasing duration of follow-up or employment up to 20 years. The rising trend did not continue after 20 years, however.

The epidemiologic studies are not adequate to draw conclusions concerning the carcinogenic potential of chlordane in exposed human populations. The cohort size and number of deaths from malignant neoplasms have been small. The turnover rate in both industries is high, record-keeping had historically been poor, and former employees are difficult to follow. The degree of exposure within the cohorts were not evaluated, and it is not possible to assess the risks of chlordane exposure independently of other chemical exposures incurred by the workers. Moreover, other confounding factors such as cigarette smoking could not be evaluated.

Many case reports of blood dyscrasia, including leukemia, have been reported in association with chlordane/heptachlor exposure (Epstein and Ozonoff, 1987). Although they cannot be used to establish the carcinogenicity of chlordane they should be noted and may represent the response of a susceptible population. Two case-control studies have estimated significantly elevated odds ratios for farmers in Nebraska and Iowa and leukemia mortality, particularly with corn production, a crop for which chlordane was registered (Blair and Thomas, 1979; Burmeister et al., 1982).

Chlordane exposure has been associated with excess hepatic neoplasms in all four mouse strains evaluated. Hepatocellular carcinoma was induced by chlordane treatment in C57BL/6 mice, a strain which does not spontaneously produce liver tumors (Becker and Sell, 1979). Malignancy was demonstrated by the growth of transplanted carcinomas in recipient mice. Chlordane also caused multiple tumors, from one to seven per mouse. Important data relative to study design were not reported, and therefore the results from this study cannot be used to evaluate dose-response. Significant dose-related increases in hepatocellular carcinoma were reported by other investigators using male and female mice, and incidence relative to controls was significantly increased at more than one dose level (NCI, 1978; IRDC, 1973). The carcinomas were observed to be metastatic to the lungs (NCI, 1977).

One of the two adequate bioassays in rats reported an increased incidence of liver adenomas and carcinomas in male F-344 rats in the highest dose group (RIASBT, 1973). The combined incidence was statistically significant. These data are supported by a significantly increased incidence of liver adenoma in female Osborne-Mendel rats in the low dose group compared to controls (NCI, 1977). A dose-related trend was not observed, but incidence may have been affected by the increased mortality that occurred in high dose females.

The positive studies of carcinogenicity in mice and rats are sufficient to place chlordane into the U.S. EPA weight of evidence classification of B2, a probable human carcinogen.

According to the U.S. EPA (1987) the available literature does not provide a comprehensive assessment of genotoxic potential for chlordane, however, minimal data requirements have been met to evaluate gene mutation, chromosomal damage, and primary DNA repair. Two acceptable studies found no evidence of gene mutation in bacterial strains. These results were supported by five survey studies of chlordane. Chlordane in a metabolically activated system was an inconclusive but presumptive positive mutagen in *Zea mays* and yeast based on the results of a survey study. When tested for gene mutation in mammalian cells, nonactivated chlordane produced evidence of mutagenicity in Chinese hamster lung cells and in L5178Y mouse cells but not in adult rat liver epithelial cells or human foreskin fibroblast cells using activated and nonactivated systems.

Chlordane did not produce a dominant lethal effect in two studies using mice. Chromosomal aberrations were not found in Chinese hamster ovary cells *in vitro* when exposed to trans-chlordane. Chlordane induced DNA repair in yeast but not in rat, mouse or hamster hepatocytes. Chlordane was classified as a presumptive but unconfirmed genotoxin for SV-40 transformed human cells. Sister chromatid exchange was induced in chlordane exposed mudminnows and in Chinese hamster ovary cells *in vitro*, but not in human lymphoid cells.

Chlordane is a promoter of liver neoplasms in B6C3F1 mice induced by DEN (Williams and Numoto, 1984) and demonstrated an ability to inhibit intercellular communication between F-344 rat liver cells in culture (Telang et al., 1982). Although much of the data concerning genotoxicity are negative, the inconclusive nature of some studies, evidence of an effect on sister chromatid exchange, and the ability of chlordane to inhibit cell-cell communication are supporting evidence of chlordane's cancer enhancing properties.

Although only scant information is available concerning the disposition, metabolism, and elimination of chlordane in the mouse, the overall handling of chlordane by mice and rats appears to be similar. The scarce data available for humans also indicate that the pattern of distribution, metabolism and elimination may be similar to rodents. There is no evidence that would support a selection between mice or rats as a more appropriate species for predicting the carcinogenic potential of chlordane in humans. Humans exhibit differences from rodents including a lesser ability to metabolize and excrete trans-nonachlor, and a longer storage of chlordane residues in human tissues.

DOSE RESPONSE ASSESSMENT

Calculation of the Reference Dose (RfD)

The liver has been identified as the target organ most sensitive to the toxic action of chlordane in chronic tests. The no-adverse-effect-level (NOAEL) selected for the calculation of the reference dose (RfD) in this report is 0.06 mg/kg/day based on the conclusions of the U.S. EPA regarding the RIASBT (1983) 130 week study conducted for the Velsicol Chemical Corporation. Dietary concentrations of 5 and 25 ppm for 130 weeks caused increased regional hepatocellular hypertrophy in female F-344 rats, while no changes were noted in the hepatic cells of females fed 1 ppm.

The confidence in the selected NOAEL is low. The NOAEL of 0.06 mg/kg/day may actually be associated with alterations in liver cells and other toxicological endpoints. The evidence that this may be true is based on three subchronic studies of chlordane exposure in rats.

1. Investigators using computer image analysis identified nuclear changes in the hepatic cells of male Long-Evans rats fed 0.1 mg/kg/day for 20 weeks with 50:50 cis- and trans-chlordane (Mahon et al., 1979; Nair et al., 1980). The authors concluded that the changes were evidence of enzyme induction which, for chlordane, may be an adverse effect because oxychlordane may be the active agent resulting in toxicity.

2. Chlordane significantly increased neuroelectrical activity and altered brain wave morphology measured by electroencephalogram in Sprague-Dawley rats fed 0.15 mg/kg/day for 42 days (Hyde and Falkenberg, 1976). Only one study has been published concerning behavioral neurotoxicology and chlordane

exposure which demonstrated effects on learning acquisition. The study used higher dose levels and treated the dams only during gestation.

3. Plasma corticosterone levels were significantly increased in 101 day old male mice and at 400 days of age in male and female mice exposed to a maternal dose of 0.16 mg/kg/day during gestation (Cranmer et al., 1978). Although the dams were fed chlordane only during gestation, their offspring were also exposed during the period of nursing.

Additional research should be conducted to evaluate these endpoints in greater depth using lower dose levels administered over a longer time period.

Table 12 presents the Reference Dose (RfD) for noncarcinogenic toxicity, and the NOAEL, uncertainty factors (UF), and modifying factor (MF) upon which it is based. The NOAEL was divided by an UF of 100 to account for the potential variability in sensitivity within humans and the potential difference in sensitivity between humans and rats. A MF of 0.7 was selected to reflect the low level of confidence in the NOAEL. Two reference doses were calculated for each study. The RfD(total) represents the dose from all exposure sources expected to result in negligible effects. The RfD(fish) represents the dose from fish obtained by sport fishing expected to result in negligible effects in humans. This was determined by subtracting the dose of chlordane due to all sources other than sport fish from the RfD(total). The dose from all other sources for chlordane is 4.4×10^{-6} mg/kg/day. This estimate is derived from the U.S. FDA market basket survey for 1981-82 (Gartrell et al., 1986) which measured residue levels in a typical adolescent male diet and the assumption that approximately 90% of the total chlordane dose is derived from food.

Table 12. Parameters Used to Calculate the Reference Dose (RfD) for Systemic Effects in Humans Exposed to Chlordane.

Reference	Target Organ	Dose mg/kg/day	End-point	Study Length weeks	UF^2	MF^3	Reference Dose mg/kg/day Total	Reference Dose mg/kg/day Fish
RIASBT, 1983	Liver	0.06	NOAEL[1]	130	10H 10A	0.7	0.00042	0.00042

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[1] NOAEL = No-Adverse-Effect-Level

[2] UF = Uncertainty factor. Uncertainty factors are used to calculate the reference dose. The no-adverse-effect-level (NOAEL) is divided by factors of 10 to account for variation in sensitivity among the members of the human population, 10H; and uncertainty in extrapolating from animal data to humans, 10A. See Table 5, Methods and Scientific Rationale for Hazard Assessment, Dose Response Assessment, and Risk Characterization for details.

[3] MF= Modifying factor. An additional uncertainty factor, greater than zero and less than or equal to 10, to further adjust for scientific uncertainties of the study.

Calculation of the Potency Factor (q_1^*) for Carcinogenicity

The U.S. EPA found that two studies were suitable for a quantitative risk estimation. The data sets involve male and female CD-1 mice (IRDA, 1973) and male and female B6C3F1 mice (NCI, 1977). The data pertinent to the calculation of the human potency factor, q_1^*, are presented in tables 13 and 14. The geometric mean of the four potency estimates was taken to arrive at an overall potency factor of 1.3 $(mg/kg/day)^{-1}$ for the general population. The most sensitive sex and strain is male CD-1 mice with a potency estimated of 4.7 $(mg/kg/day)^{-1}$. It should be noted that the potency factors are based on the number of animals with carcinoma as a percent of the number of animals histologically examined. The effective number of animals defined as the number surviving beyond the week of the first appearance of tumors is not used which would be a more appropriate comparison. The two EPA estimates were used to calculate the upper 95% cancer risks for various consumption levels and fish species in the risk characterization section.

Table 13. From U.S. EPA, 1986.

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF CHLORDANE FROM HEPATOCELLULAR CARCINOMAS IN FEMALE MICE

Compound: Analytical grade chlordane
Species, strain, sex: Mouse, CD-1, female
Body weight: 0.030 kg (assumed)
Length of experiment: 19-1/2 months
Length of exposure: 18 months
Tumor site and type: Liver, carcinoma
Route, vehicle: Oral, diet
Human potency (q_1^*): 2.98 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	0/45
5	0.65	0.052	0/61
25	3.25	0.260	32/50
50	6.50	0.520	26/37

SOURCE: IRDC, 1973a.

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF CHLORDANE FROM HEPATOCELLULAR CARCINOMAS IN MALE MICE

Compound: Analytical grade chlordane
Species, strain, sex: Mouse, CD-1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 19-1/2 months
Length of exposure: 18 months
Tumor site and type: Liver, carcinoma
Route, vehicle: Oral, diet
Human potency (q_1^*): 4.74 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	3/33
5	0.65	0.052	5/55
25	3.25	0.260	41/52
50	6.50	0.520	32/39

SOURCE: IRDC, 1973a.

Table 14. From U.S. EPA, 1986.

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF CHLORDANE FROM HEPATOCELLULAR CARCINOMAS IN FEMALE MICE

Compound:	Technical grade chlordane
Species, strain, sex:	Mouse, B6C3F1, female
Body weight:	0.030 kg (assumed)
Length of experiment:	90 weeks
Length of exposure:	80 weeks
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	0.25 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	0/19
30.1	3.9	0.31	3/47
63.8	8.3	0.66	34/49

SOURCE: NCI, 1977a.

CANCER DATA SHEET FOR DERIVATION OF POTENCY OF CHLORDANE FROM HEPATOCELLULAR CARCINOMAS IN MALE MICE

Compound:	Technical grade chlordane
Species, strain, sex:	Mouse, B6C3F1, male
Body weight:	0.030 kg (assumed)
Length of experiment:	90 weeks
Length of exposure:	80 weeks
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	0.76 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	2/18
29.9	3.9	0.31	16/48
56.2	7.3	0.58	43/49

SOURCE: NCI, 1977a.

RISK CHARACTERIZATION

Tables in the Appendix to this report present the upper 95% limit on cancer risk and hazard indices for each sport fish species using alternative values for meal size and meal frequency, and the average, minimum, and maximum tissue concentration in fish samples collected in 1985 and 1986. The tissue concentration data for both years were combined when they were available, or data from either year were used when a species was sampled in only one of those years (see exposure assessment section).

The estimates of cancer risk and other health hazards are for a 70 kg adult. It is important to note that children and lighter adults will have a higher chlordane dose rate per meal and therefore are more susceptible to health problems than these estimates indicate. For example, a 13.7 Kg (30 lb) toddler eating 12 quarter pound meals of brown trout (all sizes) per year, with have a chlordane intake of 0.000036 mg/kg/day. This dose rate is five times higher than the dose for a 70 kg adult eating the same amount.

Hazard Indices and Contributions to Uncertainty

Table 15 presents the hazard index for each species for a meal size of 0.228 kg (0.5 lb) and a consumption frequency of one meal per month. At a consumption rate of one meal per month, the hazard indices based on mean tissue concentration are below one for all species. The hazard indices based on mean or maximum tissue concentrations are under one for perch and walleye regardless of meal size or consumption frequency up to 0.34 kg meals eaten three times per week. Brown trout, chinook, and coho salmon have a hazard index based on the mean tissue concentration less than one for all meal sizes and consumption frequencies evaluated. The hazard indices for brown trout and chinook based on the maximum tissue concentrations are greater than one if 0.34 kg meals are eaten at a rate of two or three meals per week. The hazard index for coho salmon is greater than one in all meal sizes if one to three meals per week are eaten. Lake trout present some hazard based on mean tissue concentrations if 0.228 or 0.34 kg meals are eaten at a rate of two to three meals per week. Meal sizes from 0.114 to 0.34 kg present a hazard if one to several meals per month are eaten.

Tissue concentrations for chlordane were nondetectable in individual fish from samples of several species. The range in tissue concentration results in a variation in hazard indices within an order of magnitude between the maximum and minimum tissue concentrations for each species.

Upper 95% Limits on Cancer Risk Estimates and Contributions to Uncertainty

An upper 95% confidence limit for potency of 1.3 $(mg/kg/day)^{-1}$ was adopted from the geometric mean of the potency factors calculated from four data sets in male and female mice (two strains). This calculation was performed by the U.S. EPA (1986). The potency factors were based on a dose-related trend in hepatocellular carcinoma.

The potency factor based on the most sensitive sex and strain, male CD-1 mice, was 4.7 $(mg/kg/day)^{-1}$ (IRDC, 1973). The potency factor is based solely on the incidence of hepatocellular carcinoma. The potency factor based on this data set is 3.6 times greater than the potency factor based on the entire body of data available for mice.

The potency estimates were calculated using an assumed interspecies dose equivalency based on surface area. These estimates are about 14 times higher than potency factors calculated based on an assumed interspecies dose equivalency based on body weight.

The upper 95% limit on cancer risk resulting from the consumption of a 0.228 kg (0.5 lb) meal once a month or once a year are summarized for all sport fish species in Tables 16 and 17. The upper limit for cancer using average tissue concentration for a species (all size classes) ranges from about 1 x 10^{-6} for perch to 5 x 10^{-5} for lake trout when eaten once a month for a lifetime. The upper limit for cancer ranges from about 1 x 10^{-7} for perch to 5 x 10^{-6} for lake trout when eaten once a year for a lifetime. Risk estimates based on maximum tissue concentrations are about two to ten times higher. Fish species fall into the following rank order from lowest to highest risk; whitefish, perch, walleye, coho salmon, chinook salmon, brown trout and lake trout.

Tissue concentration data for certain species showed a rising trend in tissue concentration with increasing length. Therefore, it was possible, for those species, to evaluate the change in risk for different size classes. The cancer risk from the consumption of lake trout longer than 30 inches is twice that of smaller lake trout. The upper bound cancer risk from the consumption of brown trout longer than 20 inches is about twice that of 10 - 20 inch brown trout. The upper bound cancer risk from the consumption of chinook salmon longer than 30 inches is 5 times that of 20 - 30 inch chinook. Chlordane levels were not detectable in eight chinook between 10 and 20 inches in length.

The range in tissue concentration within species and size classes for which detectable levels of chlordane were obtained for all fish in the samples varies within an order of magnitude.

Tables 18, 19, and 20 present the number of meals per year that would result in an upper 95% cancer risk of 1 x 10^{-6}, 1 x 10^{-5}, and 1 x 10^{-4}. An upper limit risk of 1 x 10^{-6} is associated with the consumption of between one and 17 0.114 kg meals of brown trout, chinook salmon, coho salmon, walleye, or perch depending on the species and size class selected. Larger meals of these species could be eaten, but less frequently. No lake trout could be eaten to avoid a 10^{-6} upperbound risk level. An upper limit risk of 10^{-5} is associated with the consumption of two to seven 0.114 kg meals per year of lake trout, 24 to 10 meals per year of brown trout, 10 to 57 meals per year of chinook salmon, 24 to 28 meals per year of coho salmon, 57 meals per year of walleye, or 172 meals per year of perch depending on the size class selected. A risk level of 10^{-4} is associated with a greatly increased yearly consumption.

Table 15.

Comparison of Hazard Indices (HI) for Various Lake Michigan Sport Fish Species Consumed Once Per Month With Meal Size 0.228 Kg (0.5 lb). HI for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Hazard.

	HI Concentration		
Species (Size Class)	Mean	Max.	Min.
Lake Trout (All Sizes)	0.10	0.69	0.00
Lake Trout (10 - 20 inches)	0.06	0.16	0.00
Lake Trout (20 - 30 inches)	0.11	0.69	0.00
Lake Trout (> 30 inches)	0.20	0.25	0.15
Brown Trout (All Sizes)	0.03	0.07	0.00
Brown Trout (10 - 20 inches)	0.02	0.07	0.00
Brown Trout (20 - 30 inches)	0.04	0.07	0.01
Brown Trout (> 30 inches)	0.04	0.04	0.04
Chinook (All Sizes)	0.02	0.12	0.03
Chinook (20 - 30 inches)	0.01	0.03	0.00
Chinook (> 30 inches)	0.04	0.12	0.02
Coho (All Sizes)	0.02	0.21	0.00
Coho (20 - 30 inches)	0.02	0.21	0.00
Walleye (10 - 20 inches)	0.01	0.02	0.01
Perch (All Sizes)	0.00	0.01	0.00

Table 16.

Comparison of the Upper 95% Limit on Cancer Risk for Various Lake Michigan Sport Fish Species Consumed Once a Month With Meal Size of 0.228 Kg (0.5 lb). Risk for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Risk.

	Upper Limit (95%) on Risk q* =1.3 (Mg/Kg/Day)-1		
Species (Size Class)	Mean	Max.	Min.
Lake Trout (All Sizes)	5.43E-05	3.77E-04	0.00E+00
Lake Trout (10 - 20 inches)	3.06E-05	8.49E-05	0.00E+00
Lake Trout (20 - 30 inches)	5.85E-05	3.77E-04	0.00E+00
Lake Trout (> 30 inches)	1.10E-04	1.38E-04	8.07E-05
Brown Trout (All Sizes)	1.81E-05	3.76E-05	0.00E+00
Brown Trout (10 - 20 inches)	9.74E-06	3.62E-05	0.00E+00
Brown Trout (20 - 30 inches)	2.09E-05	3.76E-05	6.96E-06
Brown Trout (> 30 inches)	2.23E-05	2.23E-05	2.23E-05
Chinook (All Sizes)	8.35E-06	6.68E-05	1.39E-05
Chinook (20 - 30 inches)	4.18E-06	1.39E-05	0.00E+00
Chinook (> 30 inches)	2.23E-05	6.68E-05	8.35E-06
Coho (All Sizes)	8.35E-06	1.16E-04	0.00E+00
Coho (20 - 30 inches)	9.74E-06	1.16E-04	0.00E+00
Walleye (10 - 20 inches)	4.18E-06	8.35E-06	2.78E-06
Perch (All Sizes)	1.39E-06	4.18E-06	0.00E+00

Table 17.

Comparison of the Upper 95% Limit on Cancer Risk for Various Lake Michigan Sport Fish Species Consumed Once a Year With Meal Size of 0.228 Kg (0.5 lb). Risk for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Risk.

	Upper Limit (95%) on Risk q* = 16 (Mg/Kg/Day)-1		
Species (Size Class)	Mean	Max.	Min.
Lake Trout (All Sizes)	4.52E-06	3.14E-05	0.00E+00
Lake Trout (10 - 20 inches)	2.55E-06	7.08E-06	0.00E+00
Lake Trout (20 - 30 inches)	4.87E-06	3.14E-05	0.00E+00
Lake Trout (> 30 inches)	9.16E-06	1.15E-05	6.73E-06
Brown Trout (All Sizes)	1.51E-06	3.13E-06	0.00E+00
Brown Trout (10 - 20 inches)	8.12E-07	3.02E-06	0.00E+00
Brown Trout (20 - 30 inches)	1.74E-06	3.13E-06	5.80E-07
Brown Trout (> 30 inches)	1.86E-06	1.86E-06	1.86E-06
Chinook (All Sizes)	6.96E-07	5.57E-06	1.16E-06
Chinook (20 - 30 inches)	3.48E-07	1.16E-06	0.00E+00
Chinook (> 30 inches)	1.86E-06	5.57E-06	6.96E-07
Coho (All Sizes)	6.96E-07	9.63E-06	0.00E+00
Coho (20 - 30 inches)	8.12E-07	9.63E-06	0.00E+00
Walleye (All Sizes)	3.48E-07	6.96E-07	2.32E-07
Perch (All Sizes)	1.16E-07	3.48E-07	0.00E+00

Table 18.

A Comparison of Meal Frequencies Resulting in an Upper 95% Limit on Cancer Risk of 1E-06, 1E-05, or 1E-04 For a Meal Size of 0.114 Kg of Various Lake Michigan Sport Fish Species Ranked by Decreasing Level of Risk.

	Meal Frequency (/Yr)		
Species (Size Class)	Based on 1.00E-06 Risk	Based on 1.00E-05 Risk	Based on 1.00E-04 Risk
Lake Trout (All Sizes)	0.44	4.42	44.21
Lake Trout (10 - 20 inches)	0.78	7.84	78.36
Lake Trout (20 - 30 inches)	0.41	4.10	41.05
Lake Trout (> 30 inches)	0.22	2.18	21.82
Brown Trout (All Sizes)	1.33	13.26	132.62
Brown Trout (10 - 20 inches)	2.46	24.63	246.29
Brown Trout (20 - 30 inches)	1.15	11.49	114.93
Brown Trout (> 30 inches)	1.08	10.78	107.75
Chinook (All Sizes)	2.87	28.73	287.33
Chinook (20 - 30 inches)	5.75	57.47	574.67
Chinook (> 30 inches)	1.08	10.78	107.75
Coho (All Sizes)	2.87	28.73	287.33
Coho (20 - 30 inches)	2.46	24.63	246.29
Walleye (10 - 20 inches)	5.75	57.47	574.67
Perch (All Sizes)	17.24	172.40	1724.00

Table 15a.
Comparison of Hazard Indices (HI) for Various Lake Michigan Sport Fish Species Consumed Twice A Year With Meal Size 0.34 Kg (0.75 lb). HI for Mean, Maximum, and Minimum Tissue Concentration for Each Species. Species are Listed in Rank Order by Decreasing Level of Hazard.

	HI Concentration		
Species (Size Class)	Mean	Max.	Min.
Lake Trout (All Sizes)	1.29	8.93	0.00
Lake Trout (10 - 20 inches)	0.72	2.01	0.00
Lake Trout (20 - 30 inches)	1.38	8.93	0.00
Lake Trout (> 30 inches)	2.60	3.26	1.91
Brown Trout (All Sizes)	0.43	0.89	0.00
Brown Trout (10 - 20 inches)	0.23	0.86	0.00
Brown Trout (20 - 30 inches)	0.49	0.89	0.16
Brown Trout (> 30 inches)	0.53	0.53	0.53
Chinook (All Sizes)	0.20	1.58	0.33
Chinook (20 - 30 inches)	0.10	0.33	0.00
Chinook (> 30 inches)	0.53	1.58	0.20
Coho (All Sizes)	0.20	2.73	0.00
Coho (20 - 30 inches)	0.23	2.73	0.00
Walleye (10 - 20 inches)	0.10	0.20	0.07
Perch (All Sizes)	0.03	0.10	0.00

Table 19.

A Comparison of Meal Frequencies Resulting in an Upper 95% Limit on Cancer Risk of 1E-06, 1E-05, or 1E-04 For a Meal Size of 0.228 Kg of Various Lake Michigan Sport Fish Species Ranked by Decreasing Level of Risk.

Species (Size Class)	Meal Frequency (/Yr) Based on 1.00E-06 Risk	Based on 1.00E-05 Risk	Based on 1.00E-04 Risk
Lake Trout (All Sizes)	0.22	2.21	22.10
Lake Trout (10 - 20 inches)	0.39	3.92	39.18
Lake Trout (20 - 30 inches)	0.21	2.05	20.52
Lake Trout (> 30 inches)	0.11	1.09	10.91
Brown Trout (All Sizes)	0.66	6.63	66.31
Brown Trout (10 - 20 inches)	1.23	12.31	123.14
Brown Trout (20 - 30 inches)	0.57	5.75	57.47
Brown Trout (> 30 inches)	0.54	5.39	53.88
Chinook (All Sizes)	1.44	14.37	143.67
Chinook (20 - 30 inches)	2.87	28.73	287.33
Chinook (> 30 inches)	0.54	5.39	53.88
Coho (All Sizes)	1.44	14.37	143.67
Coho (20 - 30 inches)	1.23	12.31	123.14
Walleye (10 - 20 inches)	2.87	28.73	287.33
Perch (All Sizes)	8.62	86.20	862.00

Table 20.

A Comparison of Meal Frequencies Resulting in an Upper 95% Limit on Cancer Risk of 1E-06, 1E-05, or 1E-04 For a Meal Size of 0.34 Kg of Various Lake Michigan Sport Fish Species Ranked by Decreasing Level of Risk.

	Meal Frequency (/Yr)		
Species (Size Class)	Based on 1.00E-06 Risk	Based on 1.00E-05 Risk	Based on 1.00E-04 Risk
Lake Trout (All Sizes)	0.15	1.48	14.82
Lake Trout (10 - 20 inches)	0.26	2.63	26.27
Lake Trout (20 - 30 inches)	0.14	1.38	13.76
Lake Trout (> 30 inches)	0.07	0.73	7.32
Brown Trout (All Sizes)	0.44	4.45	44.46
Brown Trout (10 - 20 inches)	0.83	8.26	82.57
Brown Trout (20 - 30 inches)	0.39	3.85	38.53
Brown Trout (> 30 inches)	0.36	3.61	36.13
Chinook (All Sizes)	0.96	9.63	96.33
Chinook (20 - 30 inches)	1.93	19.27	192.67
Chinook (> 30 inches)	0.36	3.61	36.13
Coho (All Sizes)	0.96	9.63	96.33
Coho (20 - 30 inches)	0.83	8.26	82.57
Walleye (10 - 20 inches)	1.93	19.27	192.67
Perch (All Sizes)	5.78	57.80	578.00

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CHAPTER SEVEN APPENDIX

Brown Trout (10 - 20 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.07
Max.	0.26
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000001	0.000000
0.114	2	0.000625	0.000001	0.000002	0.000000
0.114	4	0.001249	0.000001	0.000005	0.000000
0.114	6	0.001874	0.000002	0.000007	0.000000
0.114	12	0.003748	0.000004	0.000014	0.000000
0.114	24	0.007496	0.000007	0.000028	0.000000
0.114	52	0.016241	0.000016	0.000060	0.000000
0.114	104	0.032482	0.000032	0.000121	0.000000
0.114	156	0.048723	0.000049	0.000181	0.000000
0.228	1	0.000625	0.000001	0.000002	0.000000
0.228	2	0.001249	0.000001	0.000005	0.000000
0.228	4	0.002499	0.000002	0.000009	0.000000
0.228	6	0.003748	0.000004	0.000014	0.000000
0.228	12	0.007496	0.000007	0.000028	0.000000
0.228	24	0.014992	0.000015	0.000056	0.000000
0.228	52	0.032482	0.000032	0.000121	0.000000
0.228	104	0.064964	0.000065	0.000241	0.000000
0.228	156	0.097447	0.000097	0.000362	0.000000
0.34	1	0.000932	0.000001	0.000003	0.000000
0.34	2	0.001863	0.000002	0.000007	0.000000
0.34	4	0.003726	0.000004	0.000014	0.000000
0.34	6	0.005589	0.000006	0.000021	0.000000
0.34	12	0.011178	0.000011	0.000042	0.000000
0.34	24	0.022356	0.000022	0.000083	0.000000
0.34	52	0.048438	0.000048	0.000180	0.000000
0.34	104	0.096877	0.000097	0.000360	0.000000
0.34	156	0.145315	0.000145	0.000540	0.000000

Upper Limit (95%) on Risk q* =1.3 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* =4.7 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
4.06E-07	1.51E-06	0.00E+00	1.47E-06	5.45E-06	0.00E+00
8.12E-07	3.02E-06	0.00E+00	2.94E-06	1.09E-05	0.00E+00
1.62E-06	6.03E-06	0.00E+00	5.87E-06	2.18E-05	0.00E+00
2.44E-06	9.05E-06	0.00E+00	8.81E-06	3.27E-05	0.00E+00
4.87E-06	1.81E-05	0.00E+00	1.76E-05	6.54E-05	0.00E+00
9.74E-06	3.62E-05	0.00E+00	3.52E-05	1.31E-04	0.00E+00
2.11E-05	7.84E-05	0.00E+00	7.63E-05	2.84E-04	0.00E+00
4.22E-05	1.57E-04	0.00E+00	1.53E-04	5.67E-04	0.00E+00
6.33E-05	2.35E-04	0.00E+00	2.29E-04	8.51E-04	0.00E+00
8.12E-07	3.02E-06	0.00E+00	2.94E-06	1.09E-05	0.00E+00
1.62E-06	6.03E-06	0.00E+00	5.87E-06	2.18E-05	0.00E+00
3.25E-06	1.21E-05	0.00E+00	1.17E-05	4.36E-05	0.00E+00
4.87E-06	1.81E-05	0.00E+00	1.76E-05	6.54E-05	0.00E+00
9.74E-06	3.62E-05	0.00E+00	3.52E-05	1.31E-04	0.00E+00
1.95E-05	7.24E-05	0.00E+00	7.05E-05	2.62E-04	0.00E+00
4.22E-05	1.57E-04	0.00E+00	1.53E-04	5.67E-04	0.00E+00
8.45E-05	3.14E-04	0.00E+00	3.05E-04	1.13E-03	0.00E+00
1.27E-04	4.71E-04	0.00E+00	4.58E-04	1.70E-03	0.00E+00
1.21E-06	4.50E-06	0.00E+00	4.38E-06	1.63E-05	0.00E+00
2.42E-06	9.00E-06	0.00E+00	8.76E-06	3.25E-05	0.00E+00
4.84E-06	1.80E-05	0.00E+00	1.75E-05	6.50E-05	0.00E+00
7.27E-06	2.70E-05	0.00E+00	2.63E-05	9.76E-05	0.00E+00
1.45E-05	5.40E-05	0.00E+00	5.25E-05	1.95E-04	0.00E+00
2.91E-05	1.08E-04	0.00E+00	1.05E-04	3.90E-04	0.00E+00
6.30E-05	2.34E-04	0.00E+00	2.28E-04	8.46E-04	0.00E+00
1.26E-04	4.68E-04	0.00E+00	4.55E-04	1.69E-03	0.00E+00
1.89E-04	7.02E-04	0.00E+00	6.83E-04	2.54E-03	0.00E+00

```
=======================================
          Hazard Index
     RfD =      0.00042 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
=======================================

        0.00        0.00        0.00
        0.00        0.01        0.00
        0.00        0.01        0.00
        0.00        0.02        0.00
        0.01        0.03        0.00
        0.02        0.07        0.00
        0.04        0.14        0.00
        0.08        0.29        0.00
        0.12        0.43        0.00

        0.00        0.01        0.00
        0.00        0.01        0.00
        0.01        0.02        0.00
        0.01        0.03        0.00
        0.02        0.07        0.00
        0.04        0.13        0.00
        0.08        0.29        0.00
        0.15        0.57        0.00
        0.23        0.86        0.00

        0.00        0.01        0.00
        0.00        0.02        0.00
        0.01        0.03        0.00
        0.01        0.05        0.00
        0.03        0.10        0.00
        0.05        0.20        0.00
        0.12        0.43        0.00
        0.23        0.86        0.00
        0.35        1.29        0.00
```

Brown Trout (20 - 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.15
Max.	0.27
Min.	0.15
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000001	0.000000
0.114	2	0.000625	0.000001	0.000002	0.000000
0.114	4	0.001249	0.000003	0.000005	0.000001
0.114	6	0.001874	0.000004	0.000007	0.000001
0.114	12	0.003748	0.000008	0.000014	0.000003
0.114	24	0.007496	0.000016	0.000029	0.000005
0.114	52	0.016241	0.000035	0.000063	0.000012
0.114	104	0.032482	0.000070	0.000125	0.000023
0.114	156	0.048723	0.000104	0.000188	0.000035
0.228	1	0.000625	0.000001	0.000002	0.000000
0.228	2	0.001249	0.000003	0.000005	0.000001
0.228	4	0.002499	0.000005	0.000010	0.000002
0.228	6	0.003748	0.000008	0.000014	0.000003
0.228	12	0.007496	0.000016	0.000029	0.000005
0.228	24	0.014992	0.000032	0.000058	0.000011
0.228	52	0.032482	0.000070	0.000125	0.000023
0.228	104	0.064964	0.000139	0.000251	0.000046
0.228	156	0.097447	0.000209	0.000376	0.000070
0.34	1	0.000932	0.000002	0.000004	0.000001
0.34	2	0.001863	0.000004	0.000007	0.000001
0.34	4	0.003726	0.000008	0.000014	0.000003
0.34	6	0.005589	0.000012	0.000022	0.000004
0.34	12	0.011178	0.000024	0.000043	0.000008
0.34	24	0.022356	0.000048	0.000086	0.000016
0.34	52	0.048438	0.000104	0.000187	0.000035
0.34	104	0.096877	0.000208	0.000374	0.000069
0.34	156	0.145315	0.000311	0.000561	0.000104

Upper Limit (95%) on Risk q* =1.3 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* =4.7 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
8.70E-07	1.57E-06	2.90E-07	3.15E-06	5.66E-06	1.05E-06
1.74E-06	3.13E-06	5.80E-07	6.29E-06	1.13E-05	2.10E-06
3.48E-06	6.26E-06	1.16E-06	1.26E-05	2.26E-05	4.19E-06
5.22E-06	9.40E-06	1.74E-06	1.89E-05	3.40E-05	6.29E-06
1.04E-05	1.88E-05	3.48E-06	3.77E-05	6.79E-05	1.26E-05
2.09E-05	3.76E-05	6.96E-06	7.55E-05	1.36E-04	2.52E-05
4.52E-05	8.14E-05	1.51E-05	1.64E-04	2.94E-04	5.45E-05
9.05E-05	1.63E-04	3.02E-05	3.27E-04	5.89E-04	1.09E-04
1.36E-04	2.44E-04	4.52E-05	4.91E-04	8.83E-04	1.64E-04
1.74E-06	3.13E-06	5.80E-07	6.29E-06	1.13E-05	2.10E-06
3.48E-06	6.26E-06	1.16E-06	1.26E-05	2.26E-05	4.19E-06
6.96E-06	1.25E-05	2.32E-06	2.52E-05	4.53E-05	8.39E-06
1.04E-05	1.88E-05	3.48E-06	3.77E-05	6.79E-05	1.26E-05
2.09E-05	3.76E-05	6.96E-06	7.55E-05	1.36E-04	2.52E-05
4.18E-05	7.52E-05	1.39E-05	1.51E-04	2.72E-04	5.03E-05
9.05E-05	1.63E-04	3.02E-05	3.27E-04	5.89E-04	1.09E-04
1.81E-04	3.26E-04	6.03E-05	6.54E-04	1.18E-03	2.18E-04
2.71E-04	4.89E-04	9.05E-05	9.81E-04	1.77E-03	3.27E-04
2.59E-06	4.67E-06	8.65E-07	9.38E-06	1.69E-05	3.13E-06
5.19E-06	9.34E-06	1.73E-06	1.88E-05	3.38E-05	6.25E-06
1.04E-05	1.87E-05	3.46E-06	3.75E-05	6.75E-05	1.25E-05
1.56E-05	2.80E-05	5.19E-06	5.63E-05	1.01E-04	1.88E-05
3.11E-05	5.61E-05	1.04E-05	1.13E-04	2.03E-04	3.75E-05
6.23E-05	1.12E-04	2.08E-05	2.25E-04	4.05E-04	7.51E-05
1.35E-04	2.43E-04	4.50E-05	4.88E-04	8.78E-04	1.63E-04
2.70E-04	4.86E-04	9.00E-05	9.76E-04	1.76E-03	3.25E-04
4.05E-04	7.29E-04	1.35E-04	1.46E-03	2.63E-03	4.88E-04

Hazard Index

RfD = 0.00042 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.00	0.00	0.00
0.00	0.01	0.00
0.01	0.01	0.00
0.01	0.02	0.00
0.02	0.03	0.01
0.04	0.07	0.01
0.08	0.15	0.03
0.17	0.30	0.06
0.25	0.45	0.08
0.00	0.01	0.00
0.01	0.01	0.00
0.01	0.02	0.00
0.02	0.03	0.01
0.04	0.07	0.01
0.08	0.14	0.03
0.17	0.30	0.06
0.33	0.60	0.11
0.50	0.89	0.17
0.00	0.01	0.00
0.01	0.02	0.00
0.02	0.03	0.01
0.03	0.05	0.01
0.06	0.10	0.02
0.11	0.21	0.04
0.25	0.44	0.08
0.49	0.89	0.16
0.74	1.33	0.25

Brown Trout (> 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.16
Max.	0.16
Min.	0.16
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000001	0.000001
0.114	2	0.000625	0.000001	0.000001	0.000001
0.114	4	0.001249	0.000003	0.000003	0.000003
0.114	6	0.001874	0.000004	0.000004	0.000004
0.114	12	0.003748	0.000009	0.000009	0.000009
0.114	24	0.007496	0.000017	0.000017	0.000017
0.114	52	0.016241	0.000037	0.000037	0.000037
0.114	104	0.032482	0.000074	0.000074	0.000074
0.114	156	0.048723	0.000111	0.000111	0.000111
0.228	1	0.000625	0.000001	0.000001	0.000001
0.228	2	0.001249	0.000003	0.000003	0.000003
0.228	4	0.002499	0.000006	0.000006	0.000006
0.228	6	0.003748	0.000009	0.000009	0.000009
0.228	12	0.007496	0.000017	0.000017	0.000017
0.228	24	0.014992	0.000034	0.000034	0.000034
0.228	52	0.032482	0.000074	0.000074	0.000074
0.228	104	0.064964	0.000148	0.000148	0.000148
0.228	156	0.097447	0.000223	0.000223	0.000223
0.34	1	0.000932	0.000002	0.000002	0.000002
0.34	2	0.001863	0.000004	0.000004	0.000004
0.34	4	0.003726	0.000009	0.000009	0.000009
0.34	6	0.005589	0.000013	0.000013	0.000013
0.34	12	0.011178	0.000026	0.000026	0.000026
0.34	24	0.022356	0.000051	0.000051	0.000051
0.34	52	0.048438	0.000111	0.000111	0.000111
0.34	104	0.096877	0.000221	0.000221	0.000221
0.34	156	0.145315	0.000332	0.000332	0.000332

Upper Limit (95%) on Risk q* =1.3 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* =4.7 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
9.28E-07	9.28E-07	9.28E-07	3.36E-06	3.36E-06	3.36E-06
1.86E-06	1.86E-06	1.86E-06	6.71E-06	6.71E-06	6.71E-06
3.71E-06	3.71E-06	3.71E-06	1.34E-05	1.34E-05	1.34E-05
5.57E-06	5.57E-06	5.57E-06	2.01E-05	2.01E-05	2.01E-05
1.11E-05	1.11E-05	1.11E-05	4.03E-05	4.03E-05	4.03E-05
2.23E-05	2.23E-05	2.23E-05	8.05E-05	8.05E-05	8.05E-05
4.83E-05	4.83E-05	4.83E-05	1.74E-04	1.74E-04	1.74E-04
9.65E-05	9.65E-05	9.65E-05	3.49E-04	3.49E-04	3.49E-04
1.45E-04	1.45E-04	1.45E-04	5.23E-04	5.23E-04	5.23E-04
1.86E-06	1.86E-06	1.86E-06	6.71E-06	6.71E-06	6.71E-06
3.71E-06	3.71E-06	3.71E-06	1.34E-05	1.34E-05	1.34E-05
7.42E-06	7.42E-06	7.42E-06	2.68E-05	2.68E-05	2.68E-05
1.11E-05	1.11E-05	1.11E-05	4.03E-05	4.03E-05	4.03E-05
2.23E-05	2.23E-05	2.23E-05	8.05E-05	8.05E-05	8.05E-05
4.45E-05	4.45E-05	4.45E-05	1.61E-04	1.61E-04	1.61E-04
9.65E-05	9.65E-05	9.65E-05	3.49E-04	3.49E-04	3.49E-04
1.93E-04	1.93E-04	1.93E-04	6.98E-04	6.98E-04	6.98E-04
2.90E-04	2.90E-04	2.90E-04	1.05E-03	1.05E-03	1.05E-03
2.77E-06	2.77E-06	2.77E-06	1.00E-05	1.00E-05	1.00E-05
5.54E-06	5.54E-06	5.54E-06	2.00E-05	2.00E-05	2.00E-05
1.11E-05	1.11E-05	1.11E-05	4.00E-05	4.00E-05	4.00E-05
1.66E-05	1.66E-05	1.66E-05	6.00E-05	6.00E-05	6.00E-05
3.32E-05	3.32E-05	3.32E-05	1.20E-04	1.20E-04	1.20E-04
6.64E-05	6.64E-05	6.64E-05	2.40E-04	2.40E-04	2.40E-04
1.44E-04	1.44E-04	1.44E-04	5.20E-04	5.20E-04	5.20E-04
2.88E-04	2.88E-04	2.88E-04	1.04E-03	1.04E-03	1.04E-03
4.32E-04	4.32E-04	4.32E-04	1.56E-03	1.56E-03	1.56E-03

```
====================================
           Hazard Index
     RfD =     0.00042 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
====================================

       0.00        0.00        0.00
       0.00        0.00        0.00
       0.01        0.01        0.01
       0.01        0.01        0.01
       0.02        0.02        0.02
       0.04        0.04        0.04
       0.09        0.09        0.09
       0.18        0.18        0.18
       0.27        0.27        0.27

       0.00        0.00        0.00
       0.01        0.01        0.01
       0.01        0.01        0.01
       0.02        0.02        0.02
       0.04        0.04        0.04
       0.08        0.08        0.08
       0.18        0.18        0.18
       0.35        0.35        0.35
       0.53        0.53        0.53

       0.01        0.01        0.01
       0.01        0.01        0.01
       0.02        0.02        0.02
       0.03        0.03        0.03
       0.06        0.06        0.06
       0.12        0.12        0.12
       0.26        0.26        0.26
       0.53        0.53        0.53
       0.79        0.79        0.79
```

Brown Trout (All Sizes)

Tissue Concentration (85 +86)	ppm
Mean	0.13
Max.	0.27
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day)		
			Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000001	0.000000
0.114	2	0.000625	0.000001	0.000002	0.000000
0.114	4	0.001249	0.000002	0.000005	0.000000
0.114	6	0.001874	0.000003	0.000007	0.000000
0.114	12	0.003748	0.000007	0.000014	0.000000
0.114	24	0.007496	0.000014	0.000029	0.000000
0.114	52	0.016241	0.000030	0.000063	0.000000
0.114	104	0.032482	0.000060	0.000125	0.000000
0.114	156	0.048723	0.000090	0.000188	0.000000
0.228	1	0.000625	0.000001	0.000002	0.000000
0.228	2	0.001249	0.000002	0.000005	0.000000
0.228	4	0.002499	0.000005	0.000010	0.000000
0.228	6	0.003748	0.000007	0.000014	0.000000
0.228	12	0.007496	0.000014	0.000029	0.000000
0.228	24	0.014992	0.000028	0.000058	0.000000
0.228	52	0.032482	0.000060	0.000125	0.000000
0.228	104	0.064964	0.000121	0.000251	0.000000
0.228	156	0.097447	0.000181	0.000376	0.000000
0.34	1	0.000932	0.000002	0.000004	0.000000
0.34	2	0.001863	0.000003	0.000007	0.000000
0.34	4	0.003726	0.000007	0.000014	0.000000
0.34	6	0.005589	0.000010	0.000022	0.000000
0.34	12	0.011178	0.000021	0.000043	0.000000
0.34	24	0.022356	0.000042	0.000086	0.000000
0.34	52	0.048438	0.000090	0.000187	0.000000
0.34	104	0.096877	0.000180	0.000374	0.000000
0.34	156	0.145315	0.000270	0.000561	0.000000

```
=========================================================================
     Upper Limit (95%) on Risk            Upper Limit (95%) on Risk
        q* =1.3 (Mg/Kg/Day)-1                q* =4.7 (Mg/Kg/Day)-1
        Mean        Max.        Min.        Mean        Max.        Min.
=========================================================================

   7.54E-07    1.57E-06    0.00E+00    2.73E-06    5.66E-06    0.00E+00
   1.51E-06    3.13E-06    0.00E+00    5.45E-06    1.13E-05    0.00E+00
   3.02E-06    6.26E-06    0.00E+00    1.09E-05    2.26E-05    0.00E+00
   4.52E-06    9.40E-06    0.00E+00    1.64E-05    3.40E-05    0.00E+00
   9.05E-06    1.88E-05    0.00E+00    3.27E-05    6.79E-05    0.00E+00
   1.81E-05    3.76E-05    0.00E+00    6.54E-05    1.36E-04    0.00E+00
   3.92E-05    8.14E-05    0.00E+00    1.42E-04    2.94E-04    0.00E+00
   7.84E-05    1.63E-04    0.00E+00    2.84E-04    5.89E-04    0.00E+00
   1.18E-04    2.44E-04    0.00E+00    4.25E-04    8.83E-04    0.00E+00

   1.51E-06    3.13E-06    0.00E+00    5.45E-06    1.13E-05    0.00E+00
   3.02E-06    6.26E-06    0.00E+00    1.09E-05    2.26E-05    0.00E+00
   6.03E-06    1.25E-05    0.00E+00    2.18E-05    4.53E-05    0.00E+00
   9.05E-06    1.88E-05    0.00E+00    3.27E-05    6.79E-05    0.00E+00
   1.81E-05    3.76E-05    0.00E+00    6.54E-05    1.36E-04    0.00E+00
   3.62E-05    7.52E-05    0.00E+00    1.31E-04    2.72E-04    0.00E+00
   7.84E-05    1.63E-04    0.00E+00    2.84E-04    5.89E-04    0.00E+00
   1.57E-04    3.26E-04    0.00E+00    5.67E-04    1.18E-03    0.00E+00
   2.35E-04    4.89E-04    0.00E+00    8.51E-04    1.77E-03    0.00E+00

   2.25E-06    4.67E-06    0.00E+00    8.13E-06    1.69E-05    0.00E+00
   4.50E-06    9.34E-06    0.00E+00    1.63E-05    3.38E-05    0.00E+00
   9.00E-06    1.87E-05    0.00E+00    3.25E-05    6.75E-05    0.00E+00
   1.35E-05    2.80E-05    0.00E+00    4.88E-05    1.01E-04    0.00E+00
   2.70E-05    5.61E-05    0.00E+00    9.76E-05    2.03E-04    0.00E+00
   5.40E-05    1.12E-04    0.00E+00    1.95E-04    4.05E-04    0.00E+00
   1.17E-04    2.43E-04    0.00E+00    4.23E-04    8.78E-04    0.00E+00
   2.34E-04    4.86E-04    0.00E+00    8.46E-04    1.76E-03    0.00E+00
   3.51E-04    7.29E-04    0.00E+00    1.27E-03    2.63E-03    0.00E+00
```

```
====================================
            Hazard Index
      RfD =     0.00042 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
====================================

       0.00        0.00        0.00
       0.00        0.01        0.00
       0.01        0.01        0.00
       0.01        0.02        0.00
       0.02        0.03        0.00
       0.03        0.07        0.00
       0.07        0.15        0.00
       0.14        0.30        0.00
       0.22        0.45        0.00

       0.00        0.01        0.00
       0.01        0.01        0.00
       0.01        0.02        0.00
       0.02        0.03        0.00
       0.03        0.07        0.00
       0.07        0.14        0.00
       0.14        0.30        0.00
       0.29        0.60        0.00
       0.43        0.89        0.00

       0.00        0.01        0.00
       0.01        0.02        0.00
       0.02        0.03        0.00
       0.02        0.05        0.00
       0.05        0.10        0.00
       0.10        0.21        0.00
       0.21        0.44        0.00
       0.43        0.89        0.00
       0.64        1.33        0.00
```

Chinook (All Sizes)

Tissue Concentration (85 +86)	ppm
Mean	0.06
Max.	0.48
Min.	0.1
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000002	0.000000
0.114	2	0.000625	0.000001	0.000004	0.000001
0.114	4	0.001249	0.000001	0.000009	0.000002
0.114	6	0.001874	0.000002	0.000013	0.000003
0.114	12	0.003748	0.000003	0.000026	0.000005
0.114	24	0.007496	0.000006	0.000051	0.000011
0.114	52	0.016241	0.000014	0.000111	0.000023
0.114	104	0.032482	0.000028	0.000223	0.000046
0.114	156	0.048723	0.000042	0.000334	0.000070
0.228	1	0.000625	0.000001	0.000004	0.000001
0.228	2	0.001249	0.000001	0.000009	0.000002
0.228	4	0.002499	0.000002	0.000017	0.000004
0.228	6	0.003748	0.000003	0.000026	0.000005
0.228	12	0.007496	0.000006	0.000051	0.000011
0.228	24	0.014992	0.000013	0.000103	0.000021
0.228	52	0.032482	0.000028	0.000223	0.000046
0.228	104	0.064964	0.000056	0.000445	0.000093
0.228	156	0.097447	0.000084	0.000668	0.000139
0.34	1	0.000932	0.000001	0.000006	0.000001
0.34	2	0.001863	0.000002	0.000013	0.000003
0.34	4	0.003726	0.000003	0.000026	0.000005
0.34	6	0.005589	0.000005	0.000038	0.000008
0.34	12	0.011178	0.000010	0.000077	0.000016
0.34	24	0.022356	0.000019	0.000153	0.000032
0.34	52	0.048438	0.000042	0.000332	0.000069
0.34	104	0.096877	0.000083	0.000664	0.000138
0.34	156	0.145315	0.000125	0.000996	0.000208

Upper Limit (95%) on Risk q* =1.3 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* =4.7 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
3.48E-07	2.78E-06	5.80E-07	1.26E-06	1.01E-05	2.10E-06
6.96E-07	5.57E-06	1.16E-06	2.52E-06	2.01E-05	4.19E-06
1.39E-06	1.11E-05	2.32E-06	5.03E-06	4.03E-05	8.39E-06
2.09E-06	1.67E-05	3.48E-06	7.55E-06	6.04E-05	1.26E-05
4.18E-06	3.34E-05	6.96E-06	1.51E-05	1.21E-04	2.52E-05
8.35E-06	6.68E-05	1.39E-05	3.02E-05	2.42E-04	5.03E-05
1.81E-05	1.45E-04	3.02E-05	6.54E-05	5.23E-04	1.09E-04
3.62E-05	2.90E-04	6.03E-05	1.31E-04	1.05E-03	2.18E-04
5.43E-05	4.34E-04	9.05E-05	1.96E-04	1.57E-03	3.27E-04
6.96E-07	5.57E-06	1.16E-06	2.52E-06	2.01E-05	4.19E-06
1.39E-06	1.11E-05	2.32E-06	5.03E-06	4.03E-05	8.39E-06
2.78E-06	2.23E-05	4.64E-06	1.01E-05	8.05E-05	1.68E-05
4.18E-06	3.34E-05	6.96E-06	1.51E-05	1.21E-04	2.52E-05
8.35E-06	6.68E-05	1.39E-05	3.02E-05	2.42E-04	5.03E-05
1.67E-05	1.34E-04	2.78E-05	6.04E-05	4.83E-04	1.01E-04
3.62E-05	2.90E-04	6.03E-05	1.31E-04	1.05E-03	2.18E-04
7.24E-05	5.79E-04	1.21E-04	2.62E-04	2.09E-03	4.36E-04
1.09E-04	8.69E-04	1.81E-04	3.93E-04	3.14E-03	6.54E-04
1.04E-06	8.30E-06	1.73E-06	3.75E-06	3.00E-05	6.25E-06
2.08E-06	1.66E-05	3.46E-06	7.51E-06	6.00E-05	1.25E-05
4.15E-06	3.32E-05	6.92E-06	1.50E-05	1.20E-04	2.50E-05
6.23E-06	4.98E-05	1.04E-05	2.25E-05	1.80E-04	3.75E-05
1.25E-05	9.96E-05	2.08E-05	4.50E-05	3.60E-04	7.51E-05
2.49E-05	1.99E-04	4.15E-05	9.01E-05	7.21E-04	1.50E-04
5.40E-05	4.32E-04	9.00E-05	1.95E-04	1.56E-03	3.25E-04
1.08E-04	8.64E-04	1.80E-04	3.90E-04	3.12E-03	6.50E-04
1.62E-04	1.30E-03	2.70E-04	5.85E-04	4.68E-03	9.76E-04

Hazard Index
RfD = 0.00042 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.00	0.01	0.00
0.00	0.01	0.00
0.00	0.02	0.00
0.00	0.03	0.01
0.01	0.06	0.01
0.02	0.12	0.03
0.03	0.27	0.06
0.07	0.53	0.11
0.10	0.80	0.17
0.00	0.01	0.00
0.00	0.02	0.00
0.01	0.04	0.01
0.01	0.06	0.01
0.02	0.12	0.03
0.03	0.24	0.05
0.07	0.53	0.11
0.13	1.06	0.22
0.20	1.59	0.33
0.00	0.02	0.00
0.00	0.03	0.01
0.01	0.06	0.01
0.01	0.09	0.02
0.02	0.18	0.04
0.05	0.36	0.08
0.10	0.79	0.16
0.20	1.58	0.33
0.30	2.37	0.49

Chinook (20 -30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.03
Max.	0.1
Min.	0
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000000	0.000000
0.114	2	0.000625	0.000000	0.000001	0.000000
0.114	4	0.001249	0.000001	0.000002	0.000000
0.114	6	0.001874	0.000001	0.000003	0.000000
0.114	12	0.003748	0.000002	0.000005	0.000000
0.114	24	0.007496	0.000003	0.000011	0.000000
0.114	52	0.016241	0.000007	0.000023	0.000000
0.114	104	0.032482	0.000014	0.000046	0.000000
0.114	156	0.048723	0.000021	0.000070	0.000000
0.228	1	0.000625	0.000000	0.000001	0.000000
0.228	2	0.001249	0.000001	0.000002	0.000000
0.228	4	0.002499	0.000001	0.000004	0.000000
0.228	6	0.003748	0.000002	0.000005	0.000000
0.228	12	0.007496	0.000003	0.000011	0.000000
0.228	24	0.014992	0.000006	0.000021	0.000000
0.228	52	0.032482	0.000014	0.000046	0.000000
0.228	104	0.064964	0.000028	0.000093	0.000000
0.228	156	0.097447	0.000042	0.000139	0.000000
0.34	1	0.000932	0.000000	0.000001	0.000000
0.34	2	0.001863	0.000001	0.000003	0.000000
0.34	4	0.003726	0.000002	0.000005	0.000000
0.34	6	0.005589	0.000002	0.000008	0.000000
0.34	12	0.011178	0.000005	0.000016	0.000000
0.34	24	0.022356	0.000010	0.000032	0.000000
0.34	52	0.048438	0.000021	0.000069	0.000000
0.34	104	0.096877	0.000042	0.000138	0.000000
0.34	156	0.145315	0.000062	0.000208	0.000000

```
=========================================================================
     Upper Limit (95%) on Risk              Upper Limit (95%) on Risk
        q* =1.3 (Mg/Kg/Day)-1                  q* =4.7 (Mg/Kg/Day)-1
      Mean           Max.          Min.       Mean           Max.          Min.
=========================================================================

   1.74E-07     5.80E-07     0.00E+00     6.29E-07     2.10E-06     0.00E+00
   3.48E-07     1.16E-06     0.00E+00     1.26E-06     4.19E-06     0.00E+00
   6.96E-07     2.32E-06     0.00E+00     2.52E-06     8.39E-06     0.00E+00
   1.04E-06     3.48E-06     0.00E+00     3.77E-06     1.26E-05     0.00E+00
   2.09E-06     6.96E-06     0.00E+00     7.55E-06     2.52E-05     0.00E+00
   4.18E-06     1.39E-05     0.00E+00     1.51E-05     5.03E-05     0.00E+00
   9.05E-06     3.02E-05     0.00E+00     3.27E-05     1.09E-04     0.00E+00
   1.81E-05     6.03E-05     0.00E+00     6.54E-05     2.18E-04     0.00E+00
   2.71E-05     9.05E-05     0.00E+00     9.81E-05     3.27E-04     0.00E+00

   3.48E-07     1.16E-06     0.00E+00     1.26E-06     4.19E-06     0.00E+00
   6.96E-07     2.32E-06     0.00E+00     2.52E-06     8.39E-06     0.00E+00
   1.39E-06     4.64E-06     0.00E+00     5.03E-06     1.68E-05     0.00E+00
   2.09E-06     6.96E-06     0.00E+00     7.55E-06     2.52E-05     0.00E+00
   4.18E-06     1.39E-05     0.00E+00     1.51E-05     5.03E-05     0.00E+00
   8.35E-06     2.78E-05     0.00E+00     3.02E-05     1.01E-04     0.00E+00
   1.81E-05     6.03E-05     0.00E+00     6.54E-05     2.18E-04     0.00E+00
   3.62E-05     1.21E-04     0.00E+00     1.31E-04     4.36E-04     0.00E+00
   5.43E-05     1.81E-04     0.00E+00     1.96E-04     6.54E-04     0.00E+00

   5.19E-07     1.73E-06     0.00E+00     1.88E-06     6.25E-06     0.00E+00
   1.04E-06     3.46E-06     0.00E+00     3.75E-06     1.25E-05     0.00E+00
   2.08E-06     6.92E-06     0.00E+00     7.51E-06     2.50E-05     0.00E+00
   3.11E-06     1.04E-05     0.00E+00     1.13E-05     3.75E-05     0.00E+00
   6.23E-06     2.08E-05     0.00E+00     2.25E-05     7.51E-05     0.00E+00
   1.25E-05     4.15E-05     0.00E+00     4.50E-05     1.50E-04     0.00E+00
   2.70E-05     9.00E-05     0.00E+00     9.76E-05     3.25E-04     0.00E+00
   5.40E-05     1.80E-04     0.00E+00     1.95E-04     6.50E-04     0.00E+00
   8.10E-05     2.70E-04     0.00E+00     2.93E-04     9.76E-04     0.00E+00
```

Hazard Index
RfD = 0.00042 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.00	0.00	0.00
0.00	0.00	0.00
0.00	0.00	0.00
0.00	0.01	0.00
0.00	0.01	0.00
0.01	0.03	0.00
0.02	0.06	0.00
0.03	0.11	0.00
0.05	0.17	0.00
0.00	0.00	0.00
0.00	0.00	0.00
0.00	0.01	0.00
0.00	0.01	0.00
0.01	0.03	0.00
0.02	0.05	0.00
0.03	0.11	0.00
0.07	0.22	0.00
0.10	0.33	0.00
0.00	0.00	0.00
0.00	0.01	0.00
0.00	0.01	0.00
0.01	0.02	0.00
0.01	0.04	0.00
0.02	0.08	0.00
0.05	0.16	0.00
0.10	0.33	0.00
0.15	0.49	0.00

Chinook (> 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.16
Max.	0.48
Min.	0.06
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000002	0.000000
0.114	2	0.000625	0.000001	0.000004	0.000001
0.114	4	0.001249	0.000003	0.000009	0.000001
0.114	6	0.001874	0.000004	0.000013	0.000002
0.114	12	0.003748	0.000009	0.000026	0.000003
0.114	24	0.007496	0.000017	0.000051	0.000006
0.114	52	0.016241	0.000037	0.000111	0.000014
0.114	104	0.032482	0.000074	0.000223	0.000028
0.114	156	0.048723	0.000111	0.000334	0.000042
0.228	1	0.000625	0.000001	0.000004	0.000001
0.228	2	0.001249	0.000003	0.000009	0.000001
0.228	4	0.002499	0.000006	0.000017	0.000002
0.228	6	0.003748	0.000009	0.000026	0.000003
0.228	12	0.007496	0.000017	0.000051	0.000006
0.228	24	0.014992	0.000034	0.000103	0.000013
0.228	52	0.032482	0.000074	0.000223	0.000028
0.228	104	0.064964	0.000148	0.000445	0.000056
0.228	156	0.097447	0.000223	0.000668	0.000084
0.34	1	0.000932	0.000002	0.000006	0.000001
0.34	2	0.001863	0.000004	0.000013	0.000002
0.34	4	0.003726	0.000009	0.000026	0.000003
0.34	6	0.005589	0.000013	0.000038	0.000005
0.34	12	0.011178	0.000026	0.000077	0.000010
0.34	24	0.022356	0.000051	0.000153	0.000019
0.34	52	0.048438	0.000111	0.000332	0.000042
0.34	104	0.096877	0.000221	0.000664	0.000083
0.34	156	0.145315	0.000332	0.000996	0.000125

Upper Limit (95%) on Risk q* =1.3 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* =4.7 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
9.28E-07	2.78E-06	3.48E-07	3.36E-06	1.01E-05	1.26E-06
1.86E-06	5.57E-06	6.96E-07	6.71E-06	2.01E-05	2.52E-06
3.71E-06	1.11E-05	1.39E-06	1.34E-05	4.03E-05	5.03E-06
5.57E-06	1.67E-05	2.09E-06	2.01E-05	6.04E-05	7.55E-06
1.11E-05	3.34E-05	4.18E-06	4.03E-05	1.21E-04	1.51E-05
2.23E-05	6.68E-05	8.35E-06	8.05E-05	2.42E-04	3.02E-05
4.83E-05	1.45E-04	1.81E-05	1.74E-04	5.23E-04	6.54E-05
9.65E-05	2.90E-04	3.62E-05	3.49E-04	1.05E-03	1.31E-04
1.45E-04	4.34E-04	5.43E-05	5.23E-04	1.57E-03	1.96E-04
1.86E-06	5.57E-06	6.96E-07	6.71E-06	2.01E-05	2.52E-06
3.71E-06	1.11E-05	1.39E-06	1.34E-05	4.03E-05	5.03E-06
7.42E-06	2.23E-05	2.78E-06	2.68E-05	8.05E-05	1.01E-05
1.11E-05	3.34E-05	4.18E-06	4.03E-05	1.21E-04	1.51E-05
2.23E-05	6.68E-05	8.35E-06	8.05E-05	2.42E-04	3.02E-05
4.45E-05	1.34E-04	1.67E-05	1.61E-04	4.83E-04	6.04E-05
9.65E-05	2.90E-04	3.62E-05	3.49E-04	1.05E-03	1.31E-04
1.93E-04	5.79E-04	7.24E-05	6.98E-04	2.09E-03	2.62E-04
2.90E-04	8.69E-04	1.09E-04	1.05E-03	3.14E-03	3.93E-04
2.77E-06	8.30E-06	1.04E-06	1.00E-05	3.00E-05	3.75E-06
5.54E-06	1.66E-05	2.08E-06	2.00E-05	6.00E-05	7.51E-06
1.11E-05	3.32E-05	4.15E-06	4.00E-05	1.20E-04	1.50E-05
1.66E-05	4.98E-05	6.23E-06	6.00E-05	1.80E-04	2.25E-05
3.32E-05	9.96E-05	1.25E-05	1.20E-04	3.60E-04	4.50E-05
6.64E-05	1.99E-04	2.49E-05	2.40E-04	7.21E-04	9.01E-05
1.44E-04	4.32E-04	5.40E-05	5.20E-04	1.56E-03	1.95E-04
2.88E-04	8.64E-04	1.08E-04	1.04E-03	3.12E-03	3.90E-04
4.32E-04	1.30E-03	1.62E-04	1.56E-03	4.68E-03	5.85E-04

Hazard Index		
RfD =	0.00042 Liver	
Mean Conc.	Max. Conc.	Min. Conc.
0.00	0.01	0.00
0.00	0.01	0.00
0.01	0.02	0.00
0.01	0.03	0.00
0.02	0.06	0.01
0.04	0.12	0.02
0.09	0.27	0.03
0.18	0.53	0.07
0.27	0.80	0.10
0.00	0.01	0.00
0.01	0.02	0.00
0.01	0.04	0.01
0.02	0.06	0.01
0.04	0.12	0.02
0.08	0.24	0.03
0.18	0.53	0.07
0.35	1.06	0.13
0.53	1.59	0.20
0.01	0.02	0.00
0.01	0.03	0.00
0.02	0.06	0.01
0.03	0.09	0.01
0.06	0.18	0.02
0.12	0.36	0.05
0.26	0.79	0.10
0.53	1.58	0.20
0.79	2.37	0.30

Coho (All Sizes)

Tissue Concentration (85 +86)	ppm
Mean	0.06
Max.	0.83
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day)		
			Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000004	0.000000
0.114	2	0.000625	0.000001	0.000007	0.000000
0.114	4	0.001249	0.000001	0.000015	0.000000
0.114	6	0.001874	0.000002	0.000022	0.000000
0.114	12	0.003748	0.000003	0.000044	0.000000
0.114	24	0.007496	0.000006	0.000089	0.000000
0.114	52	0.016241	0.000014	0.000193	0.000000
0.114	104	0.032482	0.000028	0.000385	0.000000
0.114	156	0.048723	0.000042	0.000578	0.000000
0.228	1	0.000625	0.000001	0.000007	0.000000
0.228	2	0.001249	0.000001	0.000015	0.000000
0.228	4	0.002499	0.000002	0.000030	0.000000
0.228	6	0.003748	0.000003	0.000044	0.000000
0.228	12	0.007496	0.000006	0.000089	0.000000
0.228	24	0.014992	0.000013	0.000178	0.000000
0.228	52	0.032482	0.000028	0.000385	0.000000
0.228	104	0.064964	0.000056	0.000770	0.000000
0.228	156	0.097447	0.000084	0.001155	0.000000
0.34	1	0.000932	0.000001	0.000011	0.000000
0.34	2	0.001863	0.000002	0.000022	0.000000
0.34	4	0.003726	0.000003	0.000044	0.000000
0.34	6	0.005589	0.000005	0.000066	0.000000
0.34	12	0.011178	0.000010	0.000133	0.000000
0.34	24	0.022356	0.000019	0.000265	0.000000
0.34	52	0.048438	0.000042	0.000574	0.000000
0.34	104	0.096877	0.000083	0.001149	0.000000
0.34	156	0.145315	0.000125	0.001723	0.000000

Upper Limit (95%) on Risk q* =1.3 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* =4.7 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
3.48E-07	4.81E-06	0.00E+00	1.26E-06	1.74E-05	0.00E+00
6.96E-07	9.63E-06	0.00E+00	2.52E-06	3.48E-05	0.00E+00
1.39E-06	1.93E-05	0.00E+00	5.03E-06	6.96E-05	0.00E+00
2.09E-06	2.89E-05	0.00E+00	7.55E-06	1.04E-04	0.00E+00
4.18E-06	5.78E-05	0.00E+00	1.51E-05	2.09E-04	0.00E+00
8.35E-06	1.16E-04	0.00E+00	3.02E-05	4.18E-04	0.00E+00
1.81E-05	2.50E-04	0.00E+00	6.54E-05	9.05E-04	0.00E+00
3.62E-05	5.01E-04	0.00E+00	1.31E-04	1.81E-03	0.00E+00
5.43E-05	7.51E-04	0.00E+00	1.96E-04	2.72E-03	0.00E+00
6.96E-07	9.63E-06	0.00E+00	2.52E-06	3.48E-05	0.00E+00
1.39E-06	1.93E-05	0.00E+00	5.03E-06	6.96E-05	0.00E+00
2.78E-06	3.85E-05	0.00E+00	1.01E-05	1.39E-04	0.00E+00
4.18E-06	5.78E-05	0.00E+00	1.51E-05	2.09E-04	0.00E+00
8.35E-06	1.16E-04	0.00E+00	3.02E-05	4.18E-04	0.00E+00
1.67E-05	2.31E-04	0.00E+00	6.04E-05	8.35E-04	0.00E+00
3.62E-05	5.01E-04	0.00E+00	1.31E-04	1.81E-03	0.00E+00
7.24E-05	1.00E-03	0.00E+00	2.62E-04	3.62E-03	0.00E+00
1.09E-04	1.50E-03	0.00E+00	3.93E-04	5.43E-03	0.00E+00
1.04E-06	1.44E-05	0.00E+00	3.75E-06	5.19E-05	0.00E+00
2.08E-06	2.87E-05	0.00E+00	7.51E-06	1.04E-04	0.00E+00
4.15E-06	5.74E-05	0.00E+00	1.50E-05	2.08E-04	0.00E+00
6.23E-06	8.62E-05	0.00E+00	2.25E-05	3.11E-04	0.00E+00
1.25E-05	1.72E-04	0.00E+00	4.50E-05	6.23E-04	0.00E+00
2.49E-05	3.45E-04	0.00E+00	9.01E-05	1.25E-03	0.00E+00
5.40E-05	7.47E-04	0.00E+00	1.95E-04	2.70E-03	0.00E+00
1.08E-04	1.49E-03	0.00E+00	3.90E-04	5.40E-03	0.00E+00
1.62E-04	2.24E-03	0.00E+00	5.85E-04	8.10E-03	0.00E+00

Hazard Index

RfD = 0.00042 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.00	0.01	0.00
0.00	0.02	0.00
0.00	0.04	0.00
0.00	0.05	0.00
0.01	0.11	0.00
0.02	0.21	0.00
0.03	0.46	0.00
0.07	0.92	0.00
0.10	1.38	0.00
0.00	0.02	0.00
0.00	0.04	0.00
0.01	0.07	0.00
0.01	0.11	0.00
0.02	0.21	0.00
0.03	0.42	0.00
0.07	0.92	0.00
0.13	1.83	0.00
0.20	2.75	0.00
0.00	0.03	0.00
0.00	0.05	0.00
0.01	0.11	0.00
0.01	0.16	0.00
0.02	0.32	0.00
0.05	0.63	0.00
0.10	1.37	0.00
0.20	2.73	0.00
0.30	4.10	0.00

Coho (20 - 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.07
Max.	0.83
Min.	0
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000004	0.000000
0.114	2	0.000625	0.000001	0.000007	0.000000
0.114	4	0.001249	0.000001	0.000015	0.000000
0.114	6	0.001874	0.000002	0.000022	0.000000
0.114	12	0.003748	0.000004	0.000044	0.000000
0.114	24	0.007496	0.000007	0.000089	0.000000
0.114	52	0.016241	0.000016	0.000193	0.000000
0.114	104	0.032482	0.000032	0.000385	0.000000
0.114	156	0.048723	0.000049	0.000578	0.000000
0.228	1	0.000625	0.000001	0.000007	0.000000
0.228	2	0.001249	0.000001	0.000015	0.000000
0.228	4	0.002499	0.000002	0.000030	0.000000
0.228	6	0.003748	0.000004	0.000044	0.000000
0.228	12	0.007496	0.000007	0.000089	0.000000
0.228	24	0.014992	0.000015	0.000178	0.000000
0.228	52	0.032482	0.000032	0.000385	0.000000
0.228	104	0.064964	0.000065	0.000770	0.000000
0.228	156	0.097447	0.000097	0.001155	0.000000
0.34	1	0.000932	0.000001	0.000011	0.000000
0.34	2	0.001863	0.000002	0.000022	0.000000
0.34	4	0.003726	0.000004	0.000044	0.000000
0.34	6	0.005589	0.000006	0.000066	0.000000
0.34	12	0.011178	0.000011	0.000133	0.000000
0.34	24	0.022356	0.000022	0.000265	0.000000
0.34	52	0.048438	0.000048	0.000574	0.000000
0.34	104	0.096877	0.000097	0.001149	0.000000
0.34	156	0.145315	0.000145	0.001723	0.000000

Upper Limit (95%) on Risk			Upper Limit (95%) on Risk		
q* =1.3 (Mg/Kg/Day)-1			q* =4.7 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
4.06E-07	4.81E-06	0.00E+00	1.47E-06	1.74E-05	0.00E+00
8.12E-07	9.63E-06	0.00E+00	2.94E-06	3.48E-05	0.00E+00
1.62E-06	1.93E-05	0.00E+00	5.87E-06	6.96E-05	0.00E+00
2.44E-06	2.89E-05	0.00E+00	8.81E-06	1.04E-04	0.00E+00
4.87E-06	5.78E-05	0.00E+00	1.76E-05	2.09E-04	0.00E+00
9.74E-06	1.16E-04	0.00E+00	3.52E-05	4.18E-04	0.00E+00
2.11E-05	2.50E-04	0.00E+00	7.63E-05	9.05E-04	0.00E+00
4.22E-05	5.01E-04	0.00E+00	1.53E-04	1.81E-03	0.00E+00
6.33E-05	7.51E-04	0.00E+00	2.29E-04	2.72E-03	0.00E+00
8.12E-07	9.63E-06	0.00E+00	2.94E-06	3.48E-05	0.00E+00
1.62E-06	1.93E-05	0.00E+00	5.87E-06	6.96E-05	0.00E+00
3.25E-06	3.85E-05	0.00E+00	1.17E-05	1.39E-04	0.00E+00
4.87E-06	5.78E-05	0.00E+00	1.76E-05	2.09E-04	0.00E+00
9.74E-06	1.16E-04	0.00E+00	3.52E-05	4.18E-04	0.00E+00
1.95E-05	2.31E-04	0.00E+00	7.05E-05	8.35E-04	0.00E+00
4.22E-05	5.01E-04	0.00E+00	1.53E-04	1.81E-03	0.00E+00
8.45E-05	1.00E-03	0.00E+00	3.05E-04	3.62E-03	0.00E+00
1.27E-04	1.50E-03	0.00E+00	4.58E-04	5.43E-03	0.00E+00
1.21E-06	1.44E-05	0.00E+00	4.38E-06	5.19E-05	0.00E+00
2.42E-06	2.87E-05	0.00E+00	8.76E-06	1.04E-04	0.00E+00
4.84E-06	5.74E-05	0.00E+00	1.75E-05	2.08E-04	0.00E+00
7.27E-06	8.62E-05	0.00E+00	2.63E-05	3.11E-04	0.00E+00
1.45E-05	1.72E-04	0.00E+00	5.25E-05	6.23E-04	0.00E+00
2.91E-05	3.45E-04	0.00E+00	1.05E-04	1.25E-03	0.00E+00
6.30E-05	7.47E-04	0.00E+00	2.28E-04	2.70E-03	0.00E+00
1.26E-04	1.49E-03	0.00E+00	4.55E-04	5.40E-03	0.00E+00
1.89E-04	2.24E-03	0.00E+00	6.83E-04	8.10E-03	0.00E+00

Hazard Index
RfD = 0.00042 Liver

Mean Conc.	Max. Conc.	Min. Conc.
0.00	0.01	0.00
0.00	0.02	0.00
0.00	0.04	0.00
0.00	0.05	0.00
0.01	0.11	0.00
0.02	0.21	0.00
0.04	0.46	0.00
0.08	0.92	0.00
0.12	1.38	0.00
0.00	0.02	0.00
0.00	0.04	0.00
0.01	0.07	0.00
0.01	0.11	0.00
0.02	0.21	0.00
0.04	0.42	0.00
0.08	0.92	0.00
0.15	1.83	0.00
0.23	2.75	0.00
0.00	0.03	0.00
0.00	0.05	0.00
0.01	0.11	0.00
0.01	0.16	0.00
0.03	0.32	0.00
0.05	0.63	0.00
0.12	1.37	0.00
0.23	2.73	0.00
0.35	4.10	0.00

Lake Trout (All Sizes)

Tissue Concentration (85 +86)	ppm
Mean	0.39
Max.	2.71
Min.	0
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000002	0.000012	0.000000
0.114	2	0.000625	0.000003	0.000024	0.000000
0.114	4	0.001249	0.000007	0.000048	0.000000
0.114	6	0.001874	0.000010	0.000073	0.000000
0.114	12	0.003748	0.000021	0.000145	0.000000
0.114	24	0.007496	0.000042	0.000290	0.000000
0.114	52	0.016241	0.000090	0.000629	0.000000
0.114	104	0.032482	0.000181	0.001258	0.000000
0.114	156	0.048723	0.000271	0.001886	0.000000
0.228	1	0.000625	0.000003	0.000024	0.000000
0.228	2	0.001249	0.000007	0.000048	0.000000
0.228	4	0.002499	0.000014	0.000097	0.000000
0.228	6	0.003748	0.000021	0.000145	0.000000
0.228	12	0.007496	0.000042	0.000290	0.000000
0.228	24	0.014992	0.000084	0.000580	0.000000
0.228	52	0.032482	0.000181	0.001258	0.000000
0.228	104	0.064964	0.000362	0.002515	0.000000
0.228	156	0.097447	0.000543	0.003773	0.000000
0.34	1	0.000932	0.000005	0.000036	0.000000
0.34	2	0.001863	0.000010	0.000072	0.000000
0.34	4	0.003726	0.000021	0.000144	0.000000
0.34	6	0.005589	0.000031	0.000216	0.000000
0.34	12	0.011178	0.000062	0.000433	0.000000
0.34	24	0.022356	0.000125	0.000866	0.000000
0.34	52	0.048438	0.000270	0.001875	0.000000
0.34	104	0.096877	0.000540	0.003751	0.000000
0.34	156	0.145315	0.000810	0.005626	0.000000

```
===================================================================================
      Upper Limit (95%) on Risk                 Upper Limit (95%) on Risk
         q* =1.3 (Mg/Kg/Day)-1                     q* =4.7 (Mg/Kg/Day)-1
       Mean          Max.          Min.          Mean          Max.          Min.
===================================================================================

   2.26E-06     1.57E-05     0.00E+00     8.18E-06     5.68E-05     0.00E+00
   4.52E-06     3.14E-05     0.00E+00     1.64E-05     1.14E-04     0.00E+00
   9.05E-06     6.29E-05     0.00E+00     3.27E-05     2.27E-04     0.00E+00
   1.36E-05     9.43E-05     0.00E+00     4.91E-05     3.41E-04     0.00E+00
   2.71E-05     1.89E-04     0.00E+00     9.81E-05     6.82E-04     0.00E+00
   5.43E-05     3.77E-04     0.00E+00     1.96E-04     1.36E-03     0.00E+00
   1.18E-04     8.17E-04     0.00E+00     4.25E-04     2.96E-03     0.00E+00
   2.35E-04     1.63E-03     0.00E+00     8.51E-04     5.91E-03     0.00E+00
   3.53E-04     2.45E-03     0.00E+00     1.28E-03     8.87E-03     0.00E+00

   4.52E-06     3.14E-05     0.00E+00     1.64E-05     1.14E-04     0.00E+00
   9.05E-06     6.29E-05     0.00E+00     3.27E-05     2.27E-04     0.00E+00
   1.81E-05     1.26E-04     0.00E+00     6.54E-05     4.55E-04     0.00E+00
   2.71E-05     1.89E-04     0.00E+00     9.81E-05     6.82E-04     0.00E+00
   5.43E-05     3.77E-04     0.00E+00     1.96E-04     1.36E-03     0.00E+00
   1.09E-04     7.55E-04     0.00E+00     3.93E-04     2.73E-03     0.00E+00
   2.35E-04     1.63E-03     0.00E+00     8.51E-04     5.91E-03     0.00E+00
   4.71E-04     3.27E-03     0.00E+00     1.70E-03     1.18E-02     0.00E+00
   7.06E-04     4.90E-03     0.00E+00     2.55E-03     1.77E-02     0.00E+00

   6.75E-06     4.69E-05     0.00E+00     2.44E-05     1.69E-04     0.00E+00
   1.35E-05     9.38E-05     0.00E+00     4.88E-05     3.39E-04     0.00E+00
   2.70E-05     1.88E-04     0.00E+00     9.76E-05     6.78E-04     0.00E+00
   4.05E-05     2.81E-04     0.00E+00     1.46E-04     1.02E-03     0.00E+00
   8.10E-05     5.63E-04     0.00E+00     2.93E-04     2.03E-03     0.00E+00
   1.62E-04     1.13E-03     0.00E+00     5.85E-04     4.07E-03     0.00E+00
   3.51E-04     2.44E-03     0.00E+00     1.27E-03     8.81E-03     0.00E+00
   7.02E-04     4.88E-03     0.00E+00     2.54E-03     1.76E-02     0.00E+00
   1.05E-03     7.31E-03     0.00E+00     3.81E-03     2.64E-02     0.00E+00
```

```
====================================
            Hazard Index
      RfD =        0.00042 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
====================================

       0.00        0.03        0.00
       0.01        0.06        0.00
       0.02        0.12        0.00
       0.02        0.17        0.00
       0.05        0.35        0.00
       0.10        0.69        0.00
       0.22        1.50        0.00
       0.43        2.99        0.00
       0.65        4.49        0.00

       0.01        0.06        0.00
       0.02        0.12        0.00
       0.03        0.23        0.00
       0.05        0.35        0.00
       0.10        0.69        0.00
       0.20        1.38        0.00
       0.43        2.99        0.00
       0.86        5.99        0.00
       1.29        8.98        0.00

       0.01        0.09        0.00
       0.02        0.17        0.00
       0.05        0.34        0.00
       0.07        0.52        0.00
       0.15        1.03        0.00
       0.30        2.06        0.00
       0.64        4.46        0.00
       1.29        8.93        0.00
       1.93       13.39        0.00
```

Lake Trout (10 - 20 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.22
Max.	0.61
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000001	0.000003	0.000000
0.114	2	0.000625	0.000002	0.000005	0.000000
0.114	4	0.001249	0.000004	0.000011	0.000000
0.114	6	0.001874	0.000006	0.000016	0.000000
0.114	12	0.003748	0.000012	0.000033	0.000000
0.114	24	0.007496	0.000024	0.000065	0.000000
0.114	52	0.016241	0.000051	0.000142	0.000000
0.114	104	0.032482	0.000102	0.000283	0.000000
0.114	156	0.048723	0.000153	0.000425	0.000000
0.228	1	0.000625	0.000002	0.000005	0.000000
0.228	2	0.001249	0.000004	0.000011	0.000000
0.228	4	0.002499	0.000008	0.000022	0.000000
0.228	6	0.003748	0.000012	0.000033	0.000000
0.228	12	0.007496	0.000024	0.000065	0.000000
0.228	24	0.014992	0.000047	0.000131	0.000000
0.228	52	0.032482	0.000102	0.000283	0.000000
0.228	104	0.064964	0.000204	0.000566	0.000000
0.228	156	0.097447	0.000306	0.000849	0.000000
0.34	1	0.000932	0.000003	0.000008	0.000000
0.34	2	0.001863	0.000006	0.000016	0.000000
0.34	4	0.003726	0.000012	0.000032	0.000000
0.34	6	0.005589	0.000018	0.000049	0.000000
0.34	12	0.011178	0.000035	0.000097	0.000000
0.34	24	0.022356	0.000070	0.000195	0.000000
0.34	52	0.048438	0.000152	0.000422	0.000000
0.34	104	0.096877	0.000304	0.000844	0.000000
0.34	156	0.145315	0.000457	0.001266	0.000000

```
========================================================================
      Upper Limit (95%) on Risk            Upper Limit (95%) on Risk
        q* =1.3 (Mg/Kg/Day)-1                q* =4.7 (Mg/Kg/Day)-1
         Mean          Max.          Min.         Mean          Max.          Min.
========================================================================

   1.28E-06     3.54E-06     0.00E+00     4.61E-06     1.28E-05     0.00E+00
   2.55E-06     7.08E-06     0.00E+00     9.23E-06     2.56E-05     0.00E+00
   5.10E-06     1.42E-05     0.00E+00     1.85E-05     5.12E-05     0.00E+00
   7.66E-06     2.12E-05     0.00E+00     2.77E-05     7.68E-05     0.00E+00
   1.53E-05     4.25E-05     0.00E+00     5.54E-05     1.54E-04     0.00E+00
   3.06E-05     8.49E-05     0.00E+00     1.11E-04     3.07E-04     0.00E+00
   6.64E-05     1.84E-04     0.00E+00     2.40E-04     6.65E-04     0.00E+00
   1.33E-04     3.68E-04     0.00E+00     4.80E-04     1.33E-03     0.00E+00
   1.99E-04     5.52E-04     0.00E+00     7.20E-04     2.00E-03     0.00E+00

   2.55E-06     7.08E-06     0.00E+00     9.23E-06     2.56E-05     0.00E+00
   5.10E-06     1.42E-05     0.00E+00     1.85E-05     5.12E-05     0.00E+00
   1.02E-05     2.83E-05     0.00E+00     3.69E-05     1.02E-04     0.00E+00
   1.53E-05     4.25E-05     0.00E+00     5.54E-05     1.54E-04     0.00E+00
   3.06E-05     8.49E-05     0.00E+00     1.11E-04     3.07E-04     0.00E+00
   6.13E-05     1.70E-04     0.00E+00     2.21E-04     6.14E-04     0.00E+00
   1.33E-04     3.68E-04     0.00E+00     4.80E-04     1.33E-03     0.00E+00
   2.65E-04     7.36E-04     0.00E+00     9.60E-04     2.66E-03     0.00E+00
   3.98E-04     1.10E-03     0.00E+00     1.44E-03     3.99E-03     0.00E+00

   3.81E-06     1.06E-05     0.00E+00     1.38E-05     3.82E-05     0.00E+00
   7.61E-06     2.11E-05     0.00E+00     2.75E-05     7.63E-05     0.00E+00
   1.52E-05     4.22E-05     0.00E+00     5.50E-05     1.53E-04     0.00E+00
   2.28E-05     6.33E-05     0.00E+00     8.26E-05     2.29E-04     0.00E+00
   4.57E-05     1.27E-04     0.00E+00     1.65E-04     4.58E-04     0.00E+00
   9.13E-05     2.53E-04     0.00E+00     3.30E-04     9.16E-04     0.00E+00
   1.98E-04     5.49E-04     0.00E+00     7.16E-04     1.98E-03     0.00E+00
   3.96E-04     1.10E-03     0.00E+00     1.43E-03     3.97E-03     0.00E+00
   5.94E-04     1.65E-03     0.00E+00     2.15E-03     5.95E-03     0.00E+00
```

```
====================================
           Hazard Index
     RfD =     0.00042 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
====================================

       0.00        0.01        0.00
       0.00        0.01        0.00
       0.01        0.03        0.00
       0.01        0.04        0.00
       0.03        0.08        0.00
       0.06        0.16        0.00
       0.12        0.34        0.00
       0.24        0.67        0.00
       0.36        1.01        0.00

       0.00        0.01        0.00
       0.01        0.03        0.00
       0.02        0.05        0.00
       0.03        0.08        0.00
       0.06        0.16        0.00
       0.11        0.31        0.00
       0.24        0.67        0.00
       0.49        1.35        0.00
       0.73        2.02        0.00

       0.01        0.02        0.00
       0.01        0.04        0.00
       0.03        0.08        0.00
       0.04        0.12        0.00
       0.08        0.23        0.00
       0.17        0.46        0.00
       0.36        1.01        0.00
       0.72        2.01        0.00
       1.09        3.02        0.00
```

Lake Trout (20 - 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.42
Max.	2.71
Min.	0
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000002	0.000012	0.000000
0.114	2	0.000625	0.000004	0.000024	0.000000
0.114	4	0.001249	0.000007	0.000048	0.000000
0.114	6	0.001874	0.000011	0.000073	0.000000
0.114	12	0.003748	0.000022	0.000145	0.000000
0.114	24	0.007496	0.000045	0.000290	0.000000
0.114	52	0.016241	0.000097	0.000629	0.000000
0.114	104	0.032482	0.000195	0.001258	0.000000
0.114	156	0.048723	0.000292	0.001886	0.000000
0.228	1	0.000625	0.000004	0.000024	0.000000
0.228	2	0.001249	0.000007	0.000048	0.000000
0.228	4	0.002499	0.000015	0.000097	0.000000
0.228	6	0.003748	0.000022	0.000145	0.000000
0.228	12	0.007496	0.000045	0.000290	0.000000
0.228	24	0.014992	0.000090	0.000580	0.000000
0.228	52	0.032482	0.000195	0.001258	0.000000
0.228	104	0.064964	0.000390	0.002515	0.000000
0.228	156	0.097447	0.000585	0.003773	0.000000
0.34	1	0.000932	0.000006	0.000036	0.000000
0.34	2	0.001863	0.000011	0.000072	0.000000
0.34	4	0.003726	0.000022	0.000144	0.000000
0.34	6	0.005589	0.000034	0.000216	0.000000
0.34	12	0.011178	0.000067	0.000433	0.000000
0.34	24	0.022356	0.000134	0.000866	0.000000
0.34	52	0.048438	0.000291	0.001875	0.000000
0.34	104	0.096877	0.000581	0.003751	0.000000
0.34	156	0.145315	0.000872	0.005626	0.000000

```
==========================================================================
     Upper Limit (95%) on Risk              Upper Limit (95%) on Risk
        q* =1.3 (Mg/Kg/Day)-1                  q* =4.7 (Mg/Kg/Day)-1
      Mean          Max.          Min.        Mean          Max.          Min.
==========================================================================

   2.44E-06     1.57E-05     0.00E+00     8.81E-06     5.68E-05     0.00E+00
   4.87E-06     3.14E-05     0.00E+00     1.76E-05     1.14E-04     0.00E+00
   9.74E-06     6.29E-05     0.00E+00     3.52E-05     2.27E-04     0.00E+00
   1.46E-05     9.43E-05     0.00E+00     5.28E-05     3.41E-04     0.00E+00
   2.92E-05     1.89E-04     0.00E+00     1.06E-04     6.82E-04     0.00E+00
   5.85E-05     3.77E-04     0.00E+00     2.11E-04     1.36E-03     0.00E+00
   1.27E-04     8.17E-04     0.00E+00     4.58E-04     2.96E-03     0.00E+00
   2.53E-04     1.63E-03     0.00E+00     9.16E-04     5.91E-03     0.00E+00
   3.80E-04     2.45E-03     0.00E+00     1.37E-03     8.87E-03     0.00E+00

   4.87E-06     3.14E-05     0.00E+00     1.76E-05     1.14E-04     0.00E+00
   9.74E-06     6.29E-05     0.00E+00     3.52E-05     2.27E-04     0.00E+00
   1.95E-05     1.26E-04     0.00E+00     7.05E-05     4.55E-04     0.00E+00
   2.92E-05     1.89E-04     0.00E+00     1.06E-04     6.82E-04     0.00E+00
   5.85E-05     3.77E-04     0.00E+00     2.11E-04     1.36E-03     0.00E+00
   1.17E-04     7.55E-04     0.00E+00     4.23E-04     2.73E-03     0.00E+00
   2.53E-04     1.63E-03     0.00E+00     9.16E-04     5.91E-03     0.00E+00
   5.07E-04     3.27E-03     0.00E+00     1.83E-03     1.18E-02     0.00E+00
   7.60E-04     4.90E-03     0.00E+00     2.75E-03     1.77E-02     0.00E+00

   7.27E-06     4.69E-05     0.00E+00     2.63E-05     1.69E-04     0.00E+00
   1.45E-05     9.38E-05     0.00E+00     5.25E-05     3.39E-04     0.00E+00
   2.91E-05     1.88E-04     0.00E+00     1.05E-04     6.78E-04     0.00E+00
   4.36E-05     2.81E-04     0.00E+00     1.58E-04     1.02E-03     0.00E+00
   8.72E-05     5.63E-04     0.00E+00     3.15E-04     2.03E-03     0.00E+00
   1.74E-04     1.13E-03     0.00E+00     6.30E-04     4.07E-03     0.00E+00
   3.78E-04     2.44E-03     0.00E+00     1.37E-03     8.81E-03     0.00E+00
   7.56E-04     4.88E-03     0.00E+00     2.73E-03     1.76E-02     0.00E+00
   1.13E-03     7.31E-03     0.00E+00     4.10E-03     2.64E-02     0.00E+00
```

Hazard Index RfD = 0.00042 Liver Mean Conc.	 Max. Conc.	 Min. Conc.
0.00	0.03	0.00
0.01	0.06	0.00
0.02	0.12	0.00
0.03	0.17	0.00
0.05	0.35	0.00
0.11	0.69	0.00
0.23	1.50	0.00
0.46	2.99	0.00
0.70	4.49	0.00
0.01	0.06	0.00
0.02	0.12	0.00
0.04	0.23	0.00
0.05	0.35	0.00
0.11	0.69	0.00
0.21	1.38	0.00
0.46	2.99	0.00
0.93	5.99	0.00
1.39	8.98	0.00
0.01	0.09	0.00
0.03	0.17	0.00
0.05	0.34	0.00
0.08	0.52	0.00
0.16	1.03	0.00
0.32	2.06	0.00
0.69	4.46	0.00
1.38	8.93	0.00
2.08	13.39	0.00

Lake Trout (> 30 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.79
Max.	0.99
Min.	0.58
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000004	0.000004	0.000003
0.114	2	0.000625	0.000007	0.000009	0.000005
0.114	4	0.001249	0.000014	0.000018	0.000010
0.114	6	0.001874	0.000021	0.000027	0.000016
0.114	12	0.003748	0.000042	0.000053	0.000031
0.114	24	0.007496	0.000085	0.000106	0.000062
0.114	52	0.016241	0.000183	0.000230	0.000135
0.114	104	0.032482	0.000367	0.000459	0.000269
0.114	156	0.048723	0.000550	0.000689	0.000404
0.228	1	0.000625	0.000007	0.000009	0.000005
0.228	2	0.001249	0.000014	0.000018	0.000010
0.228	4	0.002499	0.000028	0.000035	0.000021
0.228	6	0.003748	0.000042	0.000053	0.000031
0.228	12	0.007496	0.000085	0.000106	0.000062
0.228	24	0.014992	0.000169	0.000212	0.000124
0.228	52	0.032482	0.000367	0.000459	0.000269
0.228	104	0.064964	0.000733	0.000919	0.000538
0.228	156	0.097447	0.001100	0.001378	0.000807
0.34	1	0.000932	0.000011	0.000013	0.000008
0.34	2	0.001863	0.000021	0.000026	0.000015
0.34	4	0.003726	0.000042	0.000053	0.000031
0.34	6	0.005589	0.000063	0.000079	0.000046
0.34	12	0.011178	0.000126	0.000158	0.000093
0.34	24	0.022356	0.000252	0.000316	0.000185
0.34	52	0.048438	0.000547	0.000685	0.000401
0.34	104	0.096877	0.001093	0.001370	0.000803
0.34	156	0.145315	0.001640	0.002055	0.001204

Upper Limit (95%) on Risk q* =1.3 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* =4.7 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
4.58E-06	5.74E-06	3.36E-06	1.66E-05	2.08E-05	1.22E-05
9.16E-06	1.15E-05	6.73E-06	3.31E-05	4.15E-05	2.43E-05
1.83E-05	2.30E-05	1.35E-05	6.63E-05	8.30E-05	4.87E-05
2.75E-05	3.45E-05	2.02E-05	9.94E-05	1.25E-04	7.30E-05
5.50E-05	6.89E-05	4.04E-05	1.99E-04	2.49E-04	1.46E-04
1.10E-04	1.38E-04	8.07E-05	3.98E-04	4.98E-04	2.92E-04
2.38E-04	2.99E-04	1.75E-04	8.61E-04	1.08E-03	6.32E-04
4.77E-04	5.97E-04	3.50E-04	1.72E-03	2.16E-03	1.26E-03
7.15E-04	8.96E-04	5.25E-04	2.58E-03	3.24E-03	1.90E-03
9.16E-06	1.15E-05	6.73E-06	3.31E-05	4.15E-05	2.43E-05
1.83E-05	2.30E-05	1.35E-05	6.63E-05	8.30E-05	4.87E-05
3.67E-05	4.59E-05	2.69E-05	1.33E-04	1.66E-04	9.73E-05
5.50E-05	6.89E-05	4.04E-05	1.99E-04	2.49E-04	1.46E-04
1.10E-04	1.38E-04	8.07E-05	3.98E-04	4.98E-04	2.92E-04
2.20E-04	2.76E-04	1.61E-04	7.95E-04	9.97E-04	5.84E-04
4.77E-04	5.97E-04	3.50E-04	1.72E-03	2.16E-03	1.26E-03
9.53E-04	1.19E-03	7.00E-04	3.45E-03	4.32E-03	2.53E-03
1.43E-03	1.79E-03	1.05E-03	5.17E-03	6.48E-03	3.79E-03
1.37E-05	1.71E-05	1.00E-05	4.94E-05	6.19E-05	3.63E-05
2.73E-05	3.43E-05	2.01E-05	9.88E-05	1.24E-04	7.26E-05
5.47E-05	6.85E-05	4.01E-05	1.98E-04	2.48E-04	1.45E-04
8.20E-05	1.03E-04	6.02E-05	2.96E-04	3.72E-04	2.18E-04
1.64E-04	2.06E-04	1.20E-04	5.93E-04	7.43E-04	4.35E-04
3.28E-04	4.11E-04	2.41E-04	1.19E-03	1.49E-03	8.71E-04
7.11E-04	8.91E-04	5.22E-04	2.57E-03	3.22E-03	1.89E-03
1.42E-03	1.78E-03	1.04E-03	5.14E-03	6.44E-03	3.77E-03
2.13E-03	2.67E-03	1.57E-03	7.71E-03	9.66E-03	5.66E-03

```
====================================
          Hazard Index
     RfD =     0.00042 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
====================================

        0.01        0.01        0.01
        0.02        0.02        0.01
        0.03        0.04        0.02
        0.05        0.06        0.04
        0.10        0.13        0.07
        0.20        0.25        0.15
        0.44        0.55        0.32
        0.87        1.09        0.64
        1.31        1.64        0.96

        0.02        0.02        0.01
        0.03        0.04        0.02
        0.07        0.08        0.05
        0.10        0.13        0.07
        0.20        0.25        0.15
        0.40        0.50        0.30
        0.87        1.09        0.64
        1.75        2.19        1.28
        2.62        3.28        1.92

        0.03        0.03        0.02
        0.05        0.06        0.04
        0.10        0.13        0.07
        0.15        0.19        0.11
        0.30        0.38        0.22
        0.60        0.75        0.44
        1.30        1.63        0.96
        2.60        3.26        1.91
        3.90        4.89        2.87
```

Perch (All Sizes)

Tissue Concentration (85 +86)	ppm
Mean	0.01
Max.	0.03
Min.	0
Adult Body Weight (Kg)	70

Meal Size			Daily Dose Rate (Mg/Kg/Day)		
Kg	Meals/Yr.	Kg Food/Day	Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000000	0.000000
0.114	2	0.000625	0.000000	0.000000	0.000000
0.114	4	0.001249	0.000000	0.000001	0.000000
0.114	6	0.001874	0.000000	0.000001	0.000000
0.114	12	0.003748	0.000001	0.000002	0.000000
0.114	24	0.007496	0.000001	0.000003	0.000000
0.114	52	0.016241	0.000002	0.000007	0.000000
0.114	104	0.032482	0.000005	0.000014	0.000000
0.114	156	0.048723	0.000007	0.000021	0.000000
0.228	1	0.000625	0.000000	0.000000	0.000000
0.228	2	0.001249	0.000000	0.000001	0.000000
0.228	4	0.002499	0.000000	0.000001	0.000000
0.228	6	0.003748	0.000001	0.000002	0.000000
0.228	12	0.007496	0.000001	0.000003	0.000000
0.228	24	0.014992	0.000002	0.000006	0.000000
0.228	52	0.032482	0.000005	0.000014	0.000000
0.228	104	0.064964	0.000009	0.000028	0.000000
0.228	156	0.097447	0.000014	0.000042	0.000000
0.34	1	0.000932	0.000000	0.000000	0.000000
0.34	2	0.001863	0.000000	0.000001	0.000000
0.34	4	0.003726	0.000001	0.000002	0.000000
0.34	6	0.005589	0.000001	0.000002	0.000000
0.34	12	0.011178	0.000002	0.000005	0.000000
0.34	24	0.022356	0.000003	0.000010	0.000000
0.34	52	0.048438	0.000007	0.000021	0.000000
0.34	104	0.096877	0.000014	0.000042	0.000000
0.34	156	0.145315	0.000021	0.000062	0.000000

Upper Limit (95%) on Risk q* =1.3 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* =4.7 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
5.80E-08	1.74E-07	0.00E+00	2.10E-07	6.29E-07	0.00E+00
1.16E-07	3.48E-07	0.00E+00	4.19E-07	1.26E-06	0.00E+00
2.32E-07	6.96E-07	0.00E+00	8.39E-07	2.52E-06	0.00E+00
3.48E-07	1.04E-06	0.00E+00	1.26E-06	3.77E-06	0.00E+00
6.96E-07	2.09E-06	0.00E+00	2.52E-06	7.55E-06	0.00E+00
1.39E-06	4.18E-06	0.00E+00	5.03E-06	1.51E-05	0.00E+00
3.02E-06	9.05E-06	0.00E+00	1.09E-05	3.27E-05	0.00E+00
6.03E-06	1.81E-05	0.00E+00	2.18E-05	6.54E-05	0.00E+00
9.05E-06	2.71E-05	0.00E+00	3.27E-05	9.81E-05	0.00E+00
1.16E-07	3.48E-07	0.00E+00	4.19E-07	1.26E-06	0.00E+00
2.32E-07	6.96E-07	0.00E+00	8.39E-07	2.52E-06	0.00E+00
4.64E-07	1.39E-06	0.00E+00	1.68E-06	5.03E-06	0.00E+00
6.96E-07	2.09E-06	0.00E+00	2.52E-06	7.55E-06	0.00E+00
1.39E-06	4.18E-06	0.00E+00	5.03E-06	1.51E-05	0.00E+00
2.78E-06	8.35E-06	0.00E+00	1.01E-05	3.02E-05	0.00E+00
6.03E-06	1.81E-05	0.00E+00	2.18E-05	6.54E-05	0.00E+00
1.21E-05	3.62E-05	0.00E+00	4.36E-05	1.31E-04	0.00E+00
1.81E-05	5.43E-05	0.00E+00	6.54E-05	1.96E-04	0.00E+00
1.73E-07	5.19E-07	0.00E+00	6.25E-07	1.88E-06	0.00E+00
3.46E-07	1.04E-06	0.00E+00	1.25E-06	3.75E-06	0.00E+00
6.92E-07	2.08E-06	0.00E+00	2.50E-06	7.51E-06	0.00E+00
1.04E-06	3.11E-06	0.00E+00	3.75E-06	1.13E-05	0.00E+00
2.08E-06	6.23E-06	0.00E+00	7.51E-06	2.25E-05	0.00E+00
4.15E-06	1.25E-05	0.00E+00	1.50E-05	4.50E-05	0.00E+00
9.00E-06	2.70E-05	0.00E+00	3.25E-05	9.76E-05	0.00E+00
1.80E-05	5.40E-05	0.00E+00	6.50E-05	1.95E-04	0.00E+00
2.70E-05	8.10E-05	0.00E+00	9.76E-05	2.93E-04	0.00E+00

```
====================================
            Hazard Index
      RfD =     0.00042 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
====================================

       0.00        0.00        0.00
       0.00        0.00        0.00
       0.00        0.00        0.00
       0.00        0.00        0.00
       0.00        0.00        0.00
       0.00        0.01        0.00
       0.01        0.02        0.00
       0.01        0.03        0.00
       0.02        0.05        0.00

       0.00        0.00        0.00
       0.00        0.00        0.00
       0.00        0.00        0.00
       0.00        0.00        0.00
       0.00        0.01        0.00
       0.01        0.02        0.00
       0.01        0.03        0.00
       0.02        0.07        0.00
       0.03        0.10        0.00

       0.00        0.00        0.00
       0.00        0.00        0.00
       0.00        0.00        0.00
       0.00        0.01        0.00
       0.00        0.01        0.00
       0.01        0.02        0.00
       0.02        0.05        0.00
       0.03        0.10        0.00
       0.05        0.15        0.00
```

Walleye (10 - 20 inches)

Tissue Concentration (85 +86)	ppm
Mean	0.03
Max.	0.06
Min.	0.02
Adult Body Weight (Kg)	70

Meal Size Kg	Meals/Yr.	Kg Food/Day	Daily Dose Rate (Mg/Kg/Day) Mean	Max.	Min.
0.114	1	0.000312	0.000000	0.000000	0.000000
0.114	2	0.000625	0.000000	0.000001	0.000000
0.114	4	0.001249	0.000001	0.000001	0.000000
0.114	6	0.001874	0.000001	0.000002	0.000001
0.114	12	0.003748	0.000002	0.000003	0.000001
0.114	24	0.007496	0.000003	0.000006	0.000002
0.114	52	0.016241	0.000007	0.000014	0.000005
0.114	104	0.032482	0.000014	0.000028	0.000009
0.114	156	0.048723	0.000021	0.000042	0.000014
0.228	1	0.000625	0.000000	0.000001	0.000000
0.228	2	0.001249	0.000001	0.000001	0.000000
0.228	4	0.002499	0.000001	0.000002	0.000001
0.228	6	0.003748	0.000002	0.000003	0.000001
0.228	12	0.007496	0.000003	0.000006	0.000002
0.228	24	0.014992	0.000006	0.000013	0.000004
0.228	52	0.032482	0.000014	0.000028	0.000009
0.228	104	0.064964	0.000028	0.000056	0.000019
0.228	156	0.097447	0.000042	0.000084	0.000028
0.34	1	0.000932	0.000000	0.000001	0.000000
0.34	2	0.001863	0.000001	0.000002	0.000001
0.34	4	0.003726	0.000002	0.000003	0.000001
0.34	6	0.005589	0.000002	0.000005	0.000002
0.34	12	0.011178	0.000005	0.000010	0.000003
0.34	24	0.022356	0.000010	0.000019	0.000006
0.34	52	0.048438	0.000021	0.000042	0.000014
0.34	104	0.096877	0.000042	0.000083	0.000028
0.34	156	0.145315	0.000062	0.000125	0.000042

Upper Limit (95%) on Risk q* =1.3 (Mg/Kg/Day)-1			Upper Limit (95%) on Risk q* =4.7 (Mg/Kg/Day)-1		
Mean	Max.	Min.	Mean	Max.	Min.
1.74E-07	3.48E-07	1.16E-07	6.29E-07	1.26E-06	4.19E-07
3.48E-07	6.96E-07	2.32E-07	1.26E-06	2.52E-06	8.39E-07
6.96E-07	1.39E-06	4.64E-07	2.52E-06	5.03E-06	1.68E-06
1.04E-06	2.09E-06	6.96E-07	3.77E-06	7.55E-06	2.52E-06
2.09E-06	4.18E-06	1.39E-06	7.55E-06	1.51E-05	5.03E-06
4.18E-06	8.35E-06	2.78E-06	1.51E-05	3.02E-05	1.01E-05
9.05E-06	1.81E-05	6.03E-06	3.27E-05	6.54E-05	2.18E-05
1.81E-05	3.62E-05	1.21E-05	6.54E-05	1.31E-04	4.36E-05
2.71E-05	5.43E-05	1.81E-05	9.81E-05	1.96E-04	6.54E-05
3.48E-07	6.96E-07	2.32E-07	1.26E-06	2.52E-06	8.39E-07
6.96E-07	1.39E-06	4.64E-07	2.52E-06	5.03E-06	1.68E-06
1.39E-06	2.78E-06	9.28E-07	5.03E-06	1.01E-05	3.36E-06
2.09E-06	4.18E-06	1.39E-06	7.55E-06	1.51E-05	5.03E-06
4.18E-06	8.35E-06	2.78E-06	1.51E-05	3.02E-05	1.01E-05
8.35E-06	1.67E-05	5.57E-06	3.02E-05	6.04E-05	2.01E-05
1.81E-05	3.62E-05	1.21E-05	6.54E-05	1.31E-04	4.36E-05
3.62E-05	7.24E-05	2.41E-05	1.31E-04	2.62E-04	8.72E-05
5.43E-05	1.09E-04	3.62E-05	1.96E-04	3.93E-04	1.31E-04
5.19E-07	1.04E-06	3.46E-07	1.88E-06	3.75E-06	1.25E-06
1.04E-06	2.08E-06	6.92E-07	3.75E-06	7.51E-06	2.50E-06
2.08E-06	4.15E-06	1.38E-06	7.51E-06	1.50E-05	5.00E-06
3.11E-06	6.23E-06	2.08E-06	1.13E-05	2.25E-05	7.51E-06
6.23E-06	1.25E-05	4.15E-06	2.25E-05	4.50E-05	1.50E-05
1.25E-05	2.49E-05	8.30E-06	4.50E-05	9.01E-05	3.00E-05
2.70E-05	5.40E-05	1.80E-05	9.76E-05	1.95E-04	6.50E-05
5.40E-05	1.08E-04	3.60E-05	1.95E-04	3.90E-04	1.30E-04
8.10E-05	1.62E-04	5.40E-05	2.93E-04	5.85E-04	1.95E-04

```
=======================================
             Hazard Index
      RfD =       0.00042 Liver
 Mean Conc.  Max. Conc.  Min. Conc.
=======================================

        0.00        0.00        0.00
        0.00        0.00        0.00
        0.00        0.00        0.00
        0.00        0.00        0.00
        0.00        0.01        0.00
        0.01        0.02        0.01
        0.02        0.03        0.01
        0.03        0.07        0.02
        0.05        0.10        0.03

        0.00        0.00        0.00
        0.00        0.00        0.00
        0.00        0.01        0.00
        0.00        0.01        0.00
        0.01        0.02        0.01
        0.02        0.03        0.01
        0.03        0.07        0.02
        0.07        0.13        0.04
        0.10        0.20        0.07

        0.00        0.00        0.00
        0.00        0.00        0.00
        0.00        0.01        0.00
        0.01        0.01        0.00
        0.01        0.02        0.01
        0.02        0.05        0.02
        0.05        0.10        0.03
        0.10        0.20        0.07
        0.15        0.30        0.10
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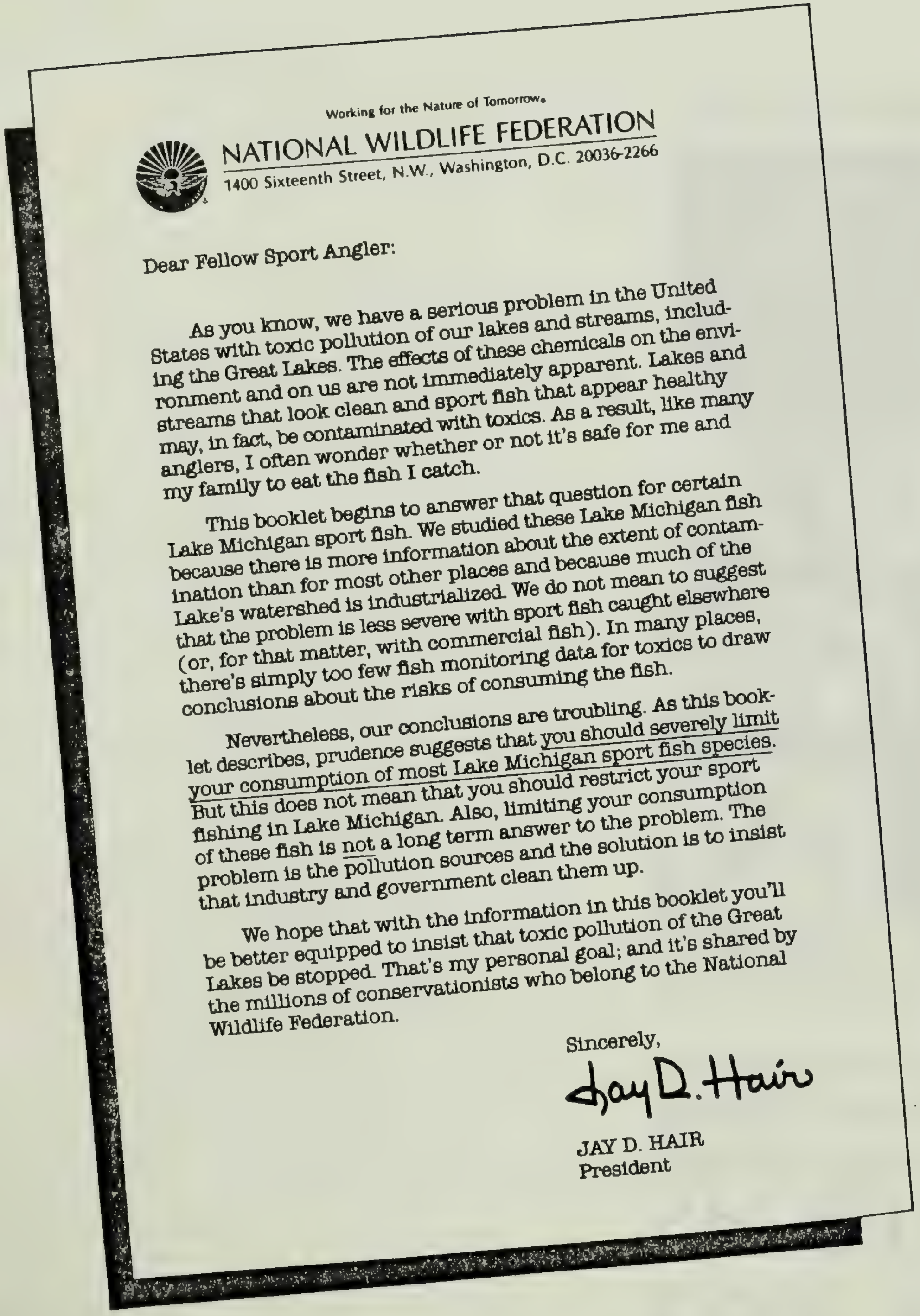
APPENDIX:

Lake Michigan Sport Fish: Should You Eat Your Catch?

LAKE MICHIGAN SPORT FISH

Should you eat your catch?



Working for the Nature of Tomorrow.
NATIONAL WILDLIFE FEDERATION
1400 Sixteenth Street, N.W., Washington, D.C. 20036-2266

Dear Fellow Sport Angler:

As you know, we have a serious problem in the United States with toxic pollution of our lakes and streams, including the Great Lakes. The effects of these chemicals on the environment and on us are not immediately apparent. Lakes and streams that look clean and sport fish that appear healthy may, in fact, be contaminated with toxics. As a result, like many anglers, I often wonder whether or not it's safe for me and my family to eat the fish I catch.

This booklet begins to answer that question for certain Lake Michigan sport fish. We studied these Lake Michigan fish because there is more information about the extent of contamination than for most other places and because much of the Lake's watershed is industrialized. We do not mean to suggest that the problem is less severe with sport fish caught elsewhere (or, for that matter, with commercial fish). In many places, there's simply too few fish monitoring data for toxics to draw conclusions about the risks of consuming the fish.

Nevertheless, our conclusions are troubling. As this booklet describes, prudence suggests that you should severely limit your consumption of most Lake Michigan sport fish species. But this does not mean that you should restrict your sport fishing in Lake Michigan. Also, limiting your consumption of these fish is not a long term answer to the problem. The problem is the pollution sources and the solution is to insist that industry and government clean them up.

We hope that with the information in this booklet you'll be better equipped to insist that toxic pollution of the Great Lakes be stopped. That's my personal goal; and it's shared by the millions of conservationists who belong to the National Wildlife Federation.

Sincerely,

Jay D. Hair

JAY D. HAIR
President

LAKE MICHIGAN
SPORT FISH

Should You Eat Your Catch?

prepared by the
NATIONAL WILDLIFE FEDERATION

Lake Michigan Sport Fish Consumption Advisory Project

Barbara S. Glenn, Project Manager
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Mark Van Putten

ACKNOWLEDGMENTS

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Many organizations and individuals assisted us during this Project by sharing information, reviewing our scientific reports, attending our April, 1989 workshop, or commenting on drafts of this booklet.

We wish to thank everyone who helped, especially those listed below, but these acknowledgments should not be misinterpreted to suggest that the conclusions in this booklet represent the views of any organizations or individuals other than the National Wildlife Federation.

- The natural resource agencies for the states of Illinois, Indiana, Michigan, and Wisconsin for providing us with the contaminant concentration data for Lake Michigan fish.

- The Project Science Review Group:

John Crellin, Ph.D.
Harold Humphrey, Ph.D.
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David Belluck, Ph.D.

- The Project Advisory Committee:

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William Davis, Wisconsin
Ralph Fritsch, Wisconsin
Frank Herres, Wisconsin
Robert Lachmund, Wisconsin
James Weishan, Wisconsin

- Kathy J. Towler, Staff Assistant, NWF Great Lakes Natural Resource Center.

All photos by Michigan Department of Natural Resources.

NWF's Lake Michigan sport fish advisory project

The Great Lakes are one of North America's most valuable resources. We rely on their sparkling waters for drinking water, for recreation, and to support our industries. Our quality of life depends on protecting the Lakes from pollution so that the waters are clean and the fish edible. Unfortunately, we have not been good stewards of these Sweetwater Seas. As a result, some Great Lakes fish are polluted with toxic chemicals that are hazardous to our health.

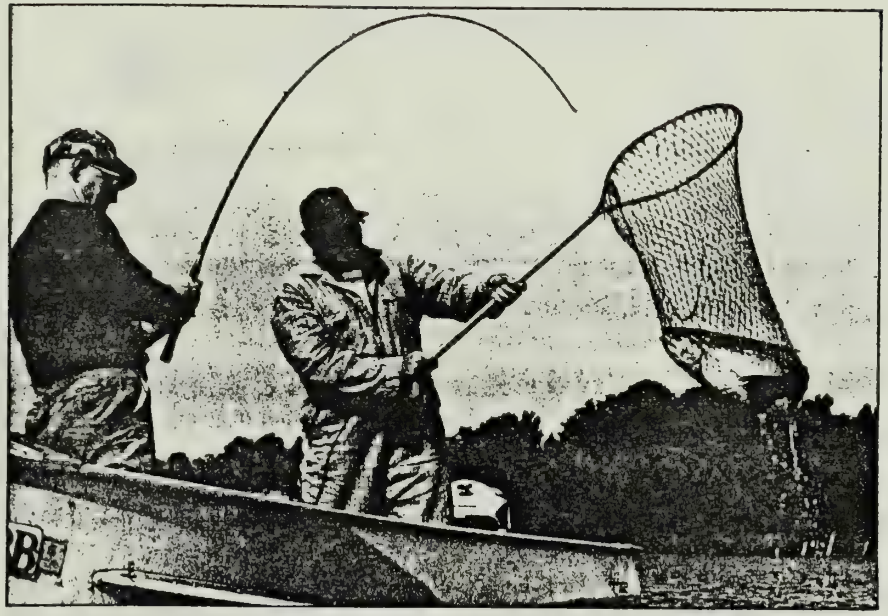
To evaluate the threat posed by consumption of Lake Michigan sport fish, NWF scientists reviewed all of the published studies of the effects of four chemicals on people and experimental animals: polychlorinated biphenyls (PCBs), DDT, dieldrin, and chlordane. DDT, dieldrin, and chlordane are pesticides that were used on crops in the Great Lakes basin. Although the U.S. and Canada have banned the use of these pesticides, they continue to enter the Great Lakes as air pollution from other countries. PCBs were used for many years by industry in a variety of ways. Most uses of PCBs have been banned. But there still is industrial dumping of PCBs into the Great Lakes watershed, and there are many areas of PCB contamination that have not been cleaned up.

These toxic substances pose a special problem because they do not break down readily in the environment. Also, small amounts of these chemicals in water can accumulate to very high levels in Great Lakes sport fish and wildlife, and to even higher levels in anglers or hunters who eat them. We limited our study to these four chemicals because there isn't enough information about the exact amounts of the other more than one hundred toxic chemicals known to contaminate Lake Michigan sport fish.

We studied the health risks from consuming lake trout, brown trout, chinook salmon, coho salmon, walleye, and yellow perch. We selected these species because they are favorites of sport anglers, and a lot of information has been collected on the amounts of the four toxic pollutants contained in these fish. Other Lake Michigan fish species such as carp and catfish are also contaminated with these and other toxic chemicals. Public health agencies have warned anglers to limit their consumption of these species.

Since the early 1970's, public health agencies have advised anglers to limit consumption of certain kinds of Lake Michigan sport fish because of toxic substances contamination. But the current consumption advisory issued by these agencies for Lake Michigan sport fish is inadequate because it's based on "action levels" for each chemical set by the Food and Drug Administration (FDA). These action levels, developed to regulate the commercial fishing industry, are outdated and do not adequately protect the health of people who eat sport fish. The FDA's action levels are based on a process that relaxes protection of public health to avoid jeopardizing the economic well-being of the commercial fishing industry. Also, the

> *We conclude . . . that the problem of toxic pollution of Lake Michigan sport fish is more serious than has previously been reported.*



Toxic pollution of the Great Lakes is an invisible threat to our health and to the health of fish and wildlife.

FDA assumes that people eat less fish – and, therefore, are exposed to less toxics – than do most Great Lakes sport anglers.

Instead of the FDA approach, NWF scientists used a "risk assessment" process to evaluate the health effects of consuming Lake Michigan sport fish. Our study is the most comprehensive evaluation ever done of the potential health effects of eating contaminated Great Lakes sport fish. In this study, NWF scientists evaluated the combined health effects that may result from eating fish containing several different chemicals, whereas other consumption advisories ignore these combined effects. A summary of NWF's study is available by writing to: NWF Lake Michigan Sport Fish Consumption Advisory Project, 802 Monroe, Ann Arbor, Michigan 48104.

We conclude, based on this study, that the problem of toxic pollution of Lake Michigan sport fish is more serious than has previously been reported. As a result, we recommend that *people should restrict their consumption of Lake Michigan sport fish even more than is currently recommended by public health agencies.*

Assessing cancer risks

The risk assessment process used by NWF to estimate cancer risks is based on studies of the number of laboratory rats and mice that developed cancer when they were fed PCBs, DDT, dieldrin, or chlordane for most of their lives. While animal tests are widely accepted in predicting whether or not a chemical will cause cancer in humans, their use in predicting how many cancers will be caused in a group of exposed people, like Lake Michigan sport anglers, is controversial. Nevertheless, the risk assessment approach we used is the best tool currently available to predict human cancer risks, and it is widely used by the U.S. Environmental Protection Agency and other regulatory agencies to develop pollution controls.

The total cancer risk from all the chemicals present in Lake Michigan sport fish may be much higher than the cancer risks we calculated for exposure to only these four toxics.

The cancer risks reported in this booklet are estimates of the odds that a person who is exposed to the mixture of these four toxic chemicals in Lake Michigan sport fish may develop cancer. In preparing these estimates, it was necessary to make certain assumptions to be sure that our lack of complete knowledge did not result in underestimating the cancer risks. For example, people may be even more sensitive to the effects of these chemicals than were the laboratory animals studied. Also, some people may be more sensitive to cancer-causing compounds than others. Our use of these assumptions is consistent with the approach widely supported by public health professionals.

As a result of these assumptions, it is unlikely that the actual cancer risks *from the four chemicals we evaluated* exceed our estimated risks, and they may be lower. On the other hand, Lake Michigan fish are contaminated with many more toxic chemicals than just PCBs, DDT, dieldrin, and chlordane. Therefore, the total cancer risk *from all the chemicals present in Lake Michigan sport fish* may be much higher than the cancer risks we calculated for exposure to only these four toxics.

In evaluating the health risks we used the most recent data (1985-86) on the levels of these toxics in fish. Despite the ban on most uses of these chemicals, the resulting decline in these contaminants in sport fish appears to have leveled off. Therefore, the 1985-86 data likely represent existing conditions. If the existing sources of these and other toxics are controlled, if the contaminated sites are cleaned up, and if the levels in Lake Michigan sport fish decline further, then the cancer risks from eating them will also decrease. That's why it's so important to clean up toxic pollution sources.

People are different and are willing to accept different risks. It is important to understand the process involved in estimating cancer risks so that *you* can use the information in this booklet to answer the question, "Should you eat your catch?"

Limiting your consumption of Lake Michigan sport fish

Lake Michigan sport fish are contaminated with many different harmful chemicals, including some that may cause cancer in humans. Over a lifetime, these cancer risks can be quite high for frequent fish eaters. Since the more contaminated fish you eat, the higher your cancer risk, you should be aware of how much and how often you eat Lake Michigan sport fish. Therefore, we recommend that you ***severely restrict your consumption of the Lake Michigan sport fish species described in this booklet.***

The cancer risk from an occasional meal of these fish is less than the risk from more frequent consumption and may be acceptable to you. The following table shows the number of meals per lifetime that are associated with a 1-in-10,000, 1-in-100,000, and 1-in-1,000,000 cancer risk (that is, of ten thousand, one hundred thousand, or one million people eating the number of fish meals indicated over their lifetime, one person may develop cancer in addition to those who would have developed cancer even if they had not eaten the fish). The table also shows the *maximum number of meals per year* that you can eat and still avoid non-cancer health problems such as liver damage.

Normally government agencies developing pollution controls would *not* allow a cancer risk as high as 1-in-10,000. Instead, they usually issue regulations to avoid an increased lifetime cancer risk higher than 1-in-100,000 or 1-in-1,000,000. The choice of an acceptable cancer risk from eating contaminated Lake Michigan sport fish is yours. But eating more than a few meals a year of most Lake Michigan sport fish over your lifetime poses high cancer risks, and we strongly recommend that you avoid eating that much fish.

> *We recommend that you severely restrict your consumption of the Lake Michigan sport fish species described in this booklet . . . women who intend to have children, pregnant women, nursing mothers, and children should not eat any of these Lake Michigan sport fish species.*

Finally, there is some special advice for children and for women who intend to have children. Young children who are not yet sexually mature may be especially sensitive to the effects of these chemicals, and exposure to cancer-causing chemicals during childhood may present an increased cancer risk compared to exposure during later periods of life. Eating these fish before and during pregnancy, even the small amounts shown in the following table, may result in subtle developmental problems in the exposed fetus and in children. Therefore, we recommend that ***women who intend to have children, pregnant women, nursing mothers, and children (15 years and younger) should not eat any of these Lake Michigan sport fish species.***

APPROXIMATE NUMBER OF LAKE MICHIGAN SPORT FISH MEALS THAT YOU CAN EAT DURING YOUR LIFETIME ASSOCIATED WITH A 1-IN-10,000, 1-IN-100,000, or 1-IN-ONE-MILLION LIFETIME RISK OF CANCER (½-pound meal size, skin-on fillets)

SPECIES/ SIZE CLASS	CANCER RISK 1-IN-10,000	1-IN-100,000	1-IN-1,000,000
Lake Trout (30" or more)	11 Meals in your lifetime (but not more than 1 meal in any one year).	1 meal in your lifetime.	No meals in your lifetime.
Lake Trout (20-30") Chinook Salmon (30" or more)	30 meals in your lifetime (but not more than 3 meals in any one year).	3 meals in your lifetime.	No meals in your lifetime.
Brown Trout (Any Size) Lake Trout (10-20") Chinook Salmon (10-30")	70 meals in your lifetime (but not more than 7 meals in any one year).	7 meals in your lifetime.	One meal in your lifetime.
Coho Salmon (Any Size) Walleye (Any Size)	150 meals in your lifetime (but not more than 17 meals in any one year).	15 meals in your lifetime.	One meal in your lifetime.
Yellow Perch (Any Size)	420 meals in your lifetime (but not more than 35 meals in any one year).	42 meals in your lifetime (but not more than 35 meals in any one year).	4 meals in your lifetime.

The maximum number of meals for any one year is shown to protect against the development of health problems other than cancer, like liver damage. The possibility of these other health problems becomes greater if the fish are eaten more frequently.

WOMEN WHO INTEND TO HAVE CHILDREN, PREGNANT WOMEN, NURSING MOTHERS, AND CHILDREN (15 YEARS AND YOUNGER) SHOULD NOT EAT ANY OF THESE LAKE MICHIGAN SPORT FISH SPECIES.

Reducing your exposure to toxic chemicals in sport fish

Whether or not you follow our recommendations to restrict consumption of Lake Michigan fish, there are other ways to reduce your current risk:

- **REDUCE THE SIZE OF EACH MEAL.**

  If ¼ pound fish meals are eaten over a 70 year lifetime instead of ½ pound meals, eating twice the number of meals shown in the preceding table would result in the same cancer risk.

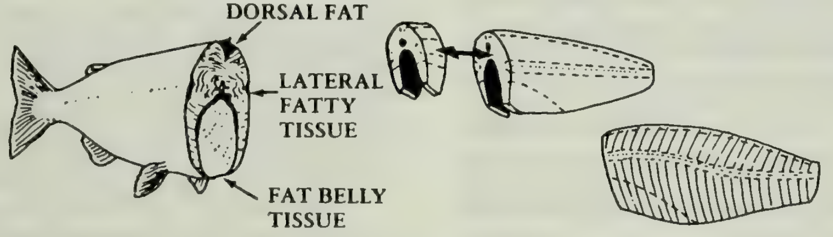
- **EAT FEWER FISH.**
- **EAT SMALLER SIZE FISH OF THE LESS CONTAMINATED SPECIES.**
- **USE PROPER PREPARATION TECHNIQUES.**

PCBs, DDT, dieldrin, and chlordane are stored in the fatty tissues of fish. Therefore, proper cleaning and cooking of your catch to remove fatty tissues may significantly reduce the amount of these chemicals. However, studies do not indicate that there is a consistent reduction in these toxics in all sizes, ages, and species of the fish we evaluated. So, while prudence dictates always properly preparing your fish, you cannot assume that it always significantly reduces your risks.

Whenever you eat Lake Michigan fish, we recommend that you always:

1. Fillet your fish and remove all internal organs.
2. Trim away fatty portions of the fish such as the dorsal, lateral, and belly area (see figure below). Trim flesh from each side of the split you make to gut the fish or remove the belly flaps of your fish fillet.
3. Remove the skin from your fish.
4. Bake, grill, or broil your fish on a rack and let fat drip away. Discard collected liquids. Avoid pan frying in butter or animal fat or making fish soups or chowders. These methods retain fat-laden juices.

While following these tips may significantly reduce your risks, they may not reduce your risks below levels generally considered to be acceptable by government regulators. The only way to reduce your cancer risks to the one-in-one-hundred-thousand or one-in-one-million level, generally deemed to be "acceptable," is to restrict your lifetime consumption to the amounts shown on the preceding page.



Trim fish as indicated by dotted lines.

Michigan Department of Public Health.

The health benefits of eating fish

Medical evidence suggests that reducing consumption of red meat and other foods high in cholesterol and saturated fat may reduce your risk of developing heart disease. Fish are lower in saturated fat and cholesterol and also have special oils that may reduce the risk of heart disease. Studies have shown that Eskimos who consume large quantities of fatty fish may have a lower incidence of heart disease as well as some other diseases.

If you are considering a dietary change to reduce your risk of coronary heart disease, we encourage you to consult your physician. If your physician recommends that you consume more fish, you should avoid fish containing high concentrations of toxic contaminants. You may want to select commercial fish caught in the open ocean, where toxic contamination levels tend to be lower. However, we do not suggest that you confine your diet to a single fish species or other food type.

Fish are lower in saturated fat and cholesterol, and also have special oils that may reduce the risk of heart disease . . . [but] you should avoid fish containing high concentrations of toxic contaminants.

The chart on the next page compares the cancer risks of eating different amounts of Lake Michigan fish and fish from two other contaminated sites (Quincy Bay, Massachusetts and Puget Sound, Washington). It also shows the cancer risks associated with eating other foods such as red meat, chicken, cod/haddock, and canned tuna. Information on contaminants in these foods was gathered from the U.S. Food and Drug Administration's Total Diet Survey for the years 1982 to 1986.

The FDA Survey reports the levels of contaminants in cooked foods, while the data for the sport fish represent raw fillets. Nevertheless, these risks can still be compared because studies have not shown that cooking consistently reduces chemical levels in fish. Moreover, even if cooking reduced the toxics in sport fish by 50%, the cancer risks from these fish would still be significantly greater than those for the commercial fish shown on this chart.

The cancer risks for these foods are based on their contamination with PCB, dieldrin, DDT, and chlordane only. These foods (as well as Great Lakes sport fish) are likely contaminated with many more harmful substances, and the risks may be higher than shown in this chart. Our estimate of the risks from eating these foods only accounts for cancer risks and does not address other risks such as heart disease.

Cancer risks should be interpreted as follows: 1 in 10 means that one person in ten exposed persons may develop cancer if they eat this food at the specified number of meals per week, month, or year for a lifetime; 1 in 100 means that one person in one hundred may develop cancer if they eat this food at the specified rate for a lifetime; 1 in 1000 means that one person in one thousand may develop cancer, and so on.

COMPARATIVE CANCER RISKS FOR LAKE MICHIGAN FISH AND OTHER FOODS

	1 meal/week	1 meal/month	1 meal/year	
Very high cancer risk				Very high cancer risk
1 in 10				1 in 10
	Lake Trout (>30 inches) Lake Trout (20-30 inches)			
1 in 100				1 in 100
	Brown Trout (all sizes) Lake Trout (10-20 inches) Chinook Salmon (all sizes) Coho Salmon (20-30 inches) Walleye (all sizes)	Lake Trout (>30 inches) Lake Trout (20-30 inches) Brown Trout (all sizes)		
1 in 1000				1 in 1000
	Flounder (Quincy Bay, MA) Pacific Cod (Puget Sound, WA) Perch (all sizes) Lobster (Quincy Bay, MA) Chinook Salmon (Puget Sound, WA)	Lake Trout (10-20 inches) Chinook Salmon (all sizes) Coho Salmon (20-30 inches) Walleye (all sizes) Flounder (Quincy Bay, MA) Pacific Cod (Puget Sound, WA) Perch (all sizes)	Lake Trout (>30 inches) Lake Trout (20-30 inches) Brown Trout (all sizes)	
1 in 10,000				1 in 10,000
		Lobster (Quincy Bay, MA) Chinook Salmon (Puget Sound, WA)	Lake Trout (10-20 inches) Chinook Salmon (all sizes) Coho Salmon (20-30 inches) Walleye (all sizes) Flounder (Quincy Bay, MA) Pacific Cod (Puget Sound, WA)	
1 in 100,000				1 in 100,000
	Red Meat Cod/Haddock Chicken		Perch (all sizes) Lobster (Quincy Bay, MA) Chinook Salmon (Puget Sound, WA)	
1 in 1,000,000				1 in 1,000,000
	Canned Tuna	Red Meat		
1 in 10,000,000				1 in 10,000,000
Very low cancer risk		Cod/Haddock Chicken Canned Tuna	Red Meat Cod/Haddock Chicken Canned Tuna	Very low cancer risk

Health hazards of PCBs, DDT, dieldrin, and chlordane

It is difficult to say exactly what health effects you may experience if you regularly eat Lake Michigan sport fish over a long period of time. This is because few studies have carefully looked at sport fish eaters. However, eating large amounts of these fish may seriously threaten your health. Most people in the United States carry small amounts of PCBs, DDT, dieldrin, and chlordane in their bodies. Much greater amounts have been found in people with known sources of exposure, including chemical manufacturing workers and *sport anglers* who regularly eat contaminated fish.

PCBs, DDT, dieldrin, and chlordane have been very harmful to people exposed to large amounts of these substances during accidental poisonings or suicide attempts. Workers exposed to these chemicals over long periods have also shown negative effects, including severe skin rashes, increased blood levels of fat and cholesterol, potential changes in liver function, and cancer.

Long-term experiments with animals provide some clues about how people may react when exposed to these chemicals. PCBs, DDT, dieldrin, and chlordane caused cancer, liver damage, disorders of the immune system, damage to the endocrine system (which regulates the body's hormones), and changes in behavior and learning ability in rodents and monkeys exposed to these chemicals.

One of the most important problems caused by exposure to PCBs, DDT, dieldrin, and chlordane is effects on reproduction. These chemicals can be passed easily in the bloodstream from the mother to the developing fetus and can be transferred to nursing infants in breast milk. It is especially troubling that

Children and women expecting to have children should not eat contaminated fish.

researchers have found several abnormalities in the children of mothers who were exposed to these chemicals by eating Lake Michigan sport fish before and during their pregnancy.

For example, one study evaluated the health of infants born to Michigan women who had eaten an average of one to two lake trout meals per month from Lake Michigan for a large part of their lives. The women gave birth to children with lower birth weights, with smaller head circumferences, and who were less mature than infants of mothers who had not eaten Lake Michigan sport fish. Motor skills were less developed in the exposed children, and, when they were seven months old, these children did less well on a learning ability test than did their nonexposed counterparts.

One of the most important problems caused by exposure to PCBs, DDT, dieldrin, and chlordane is effects on reproduction.

Although the learning differences observed in this study were not large, the children exposed to the pollutants in Lake Michigan fish were put at a disadvantage compared to other children at the very beginning of their lives. The same types of developmental problems were reported in the children of Korean and Japanese women who used cooking oil contaminated with PCBs and the children of North Carolina women with environmental exposure to PCBs and DDT. While no one study by itself proves that PCBs cause reproductive problems in people, the same problems were seen in several different studies, suggesting cause for concern.

Also, similar effects have been observed in experimental animals fed PCBS, DDT, dieldrin, and chlordane. Monkeys fed PCBs had offspring with lower birth weights, slowed weight gain, and abnormal behavior. Rats and mice fed the chemicals had smaller numbers of litters and smaller litter sizes. The exposed offspring gained weight more slowly and were generally unhealthy. Other effects in the offspring of rats and mice included liver toxicity, immune suppression, changes in the function of the endocrine system, and altered behavioral responses.

Reproduction in men may also be affected by these chemicals. A few studies using experimental animals showed that these chemicals reduced male fertility by decreasing the number of sperm cells produced and by decreasing their mobility.

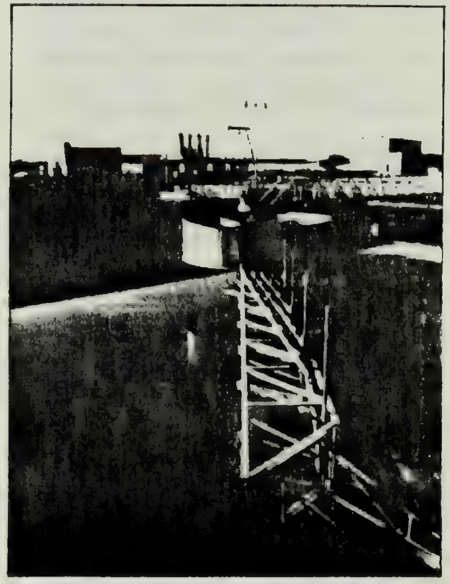
There is a great deal of evidence that exposure to PCBs, DDT, dieldrin, and chlordane causes cancer in laboratory animals. There is also evidence that these chemicals may cause cancer in people. The human evidence comes from studies of workers exposed to PCBs, and studies of pesticide manufacturing workers and pesticide control workers exposed to DDT, dieldrin, and chlordane.

Cancer of the liver and related systems is a consistent finding in studies of human exposure to PCBs. However, a direct cause-and-effect link between PCB exposure and cancer in humans has not been established with complete certainty. On the other hand, there is enough evidence from studies in experimental animals to conclude that PCBs, DDT, dieldrin, and chlordane probably cause cancer in humans.

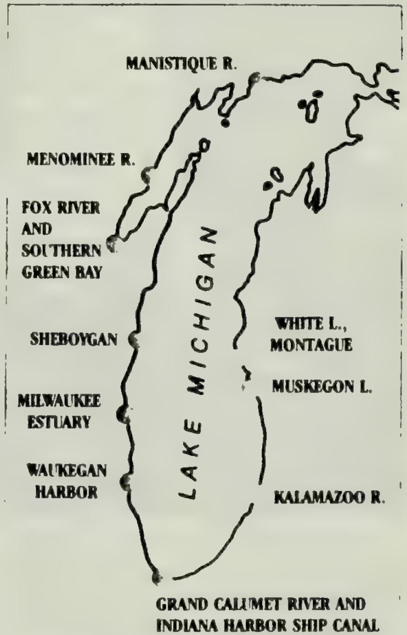
The sources of toxic pollution

Eating fewer Lake Michigan fish does not necessarily mean that you should go fishing less often. The joys of fishing and the beauty of Lake Michigan are reasons enough. The long-term solution to the problem of contaminated fish is not to stop fishing; it is to stop the toxic pollution of the Lake.

The toxic pollutants in Lake Michigan fish come from many different sources, including industrial and municipal wastewater, runoff from city streets and farm fields, polluted groundwater, river and harbor sediments, and even the rain and snow. Although government efforts to control the sources of a few Great Lakes pollutants have met with some success, much more needs to be done. For example, although many toxics enter the Great Lakes by contaminating the rain and snow, there is no federal law controlling these toxic air pollutants.



Discharges from municipal and industrial wastewater treatment plants.



RIVER AND HARBOR MOUTHS IN LAKE MICHIGAN WITH HIGH LEVELS OF CONTAMINANTS, ESPECIALLY PCBs.

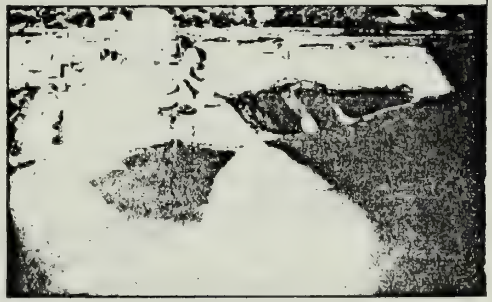
This map of Lake Michigan shows the "toxic hot spots" that have been identified by the Canadian-U.S. Great Lakes Water Quality Board. These are the most polluted areas of Lake Michigan, and they contribute significantly to the contamination of the sport fish. Government agencies are currently developing "remedial action plans" to clean up these hot spots. But those plans won't make a dif-

Pesticide runoff from agricultural fields.

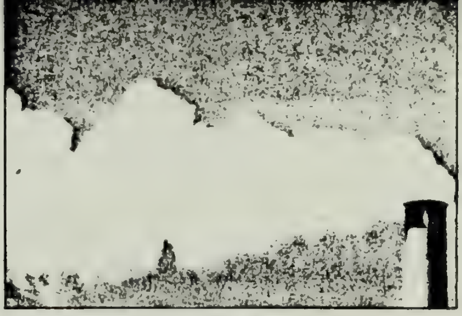
ference without the commitment and money to implement them.

Hundreds of toxics contaminate Lake Michigan and its fish and wildlife in addition to the four pollutants considered by NWF in preparing this booklet. More information is needed about the amounts of these other pollutants poisoning Lake Michigan fish. That information should be gathered at once and evaluated to determine the risks posed by these chemicals. Our federal and state government agencies must then identify and control the sources of these poisons. In the meantime, no *increased* pollution of Lake Michigan with these harmful chemicals should be allowed.

People who eat Lake Michigan sport fish have the most at stake in cleaning up the Lake. But *all* of us are a part of the Great Lakes ecosystem, and our well-being depends on the health of the Great Lakes. We hope that by knowing more about the invisible risks of toxic pollution you will become a more active citizen of the Great Lakes Basin. We want you to join us and thousands of other citizen activists in demanding that the pollution of these magnificent Sweetwater Seas be stopped. Everyone can help by contacting government officials and urging:



Dumping of liquid industrial wastes.



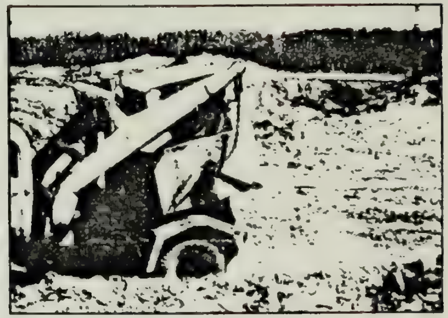
Toxic fallout from the air.

Lake and river sediments contaminated with toxics.

(1) regular testing of Lake Michigan sport fish;

(2) evaluation of those tests and issuance of consumption advisories based on state-of-the-art risk assessment techniques;

(3) controls on all sources of these toxics to reduce and then eliminate them so that unlimited amounts of Great Lakes fish can be eaten and advisories are no longer necessary.



Runoff from landfills and dumps.

> *All of us are a part of the Great Lakes ecosystem and our well-being depends on the health of the Great Lakes .*

On the last page of this booklet, you will find a list of the key government officials responsible for Lake Michigan. Write them and your federal and state legislators, too. And, if you want more information about how you can help clean up Lake Michigan, write the National Wildlife Federation's Great Lakes office at 802 Monroe, Ann Arbor, MI 48104.

If you want to know more…

About the NWF Lake Michigan Sport Fish Consumption Advisory Project:

Write NWF Fish Advisory Project, 802 Monroe, Ann Arbor, Michigan 48104 for a copy of the Project Scientific Summary or for a copy of a 15 minute VCR tape describing the Project and its conclusions. (Please include a check for $10.00 to cover the costs of duplicating and mailing the tape.)

About Great Lakes Pollution:

William Ashworth, *The Late Great Lakes*

International Joint Commission, Great Lakes Water Quality Board, *1987 Report on Great Lakes Water Quality*. This and other reports are available from the:

International Joint Commission
100 Ouellette Avenue, 8th Floor
Windsor, Ontario, Canada N9A 6T3

Wayne Schmidt. "Are Great Lakes Fish Safe To Eat?," *National Wildlife Magazine*, July/August 1989

About Toxic Chemicals & Risk Assessment:

William Lowrance, *Of Acceptable Risk*

National Wildlife Federation, *The Toxic Substances Dilemma: A Plan for Citizen Action*

About Remedial Action Plans for the "toxic hot spots":
write to:
Carol Finch, Director
Great Lakes National Program Office
U.S. EPA
230 S. Dearborn
Chicago, IL 60604

About Great Lakes Sportfishing:
Tom Huggler, *Fish Michigan – Great Lakes* stock #150
Send $18.95 plus 4% sales tax
(MI residents only) add $2.00
shipping to: MUCC Sportsman
Library Box 30235, Lansing, MI 48904

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If you want to help clean up Lake Michigan…

Write:

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Washington, D.C. 20460

U.S. EPA Great Lakes Program Manager
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Illinois Governor James Thompson
State Capitol
Springfield, Illinois 62706

Illinois EPA Director Bernard Killian
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Indiana DNR Director Patrick Ralston
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Wisconsin Governor Tommy Thompson
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Wisconsin DNR Secretary
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Wisconsin Department of
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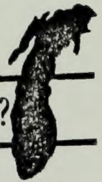
LAKE MICHIGAN SPORT FISH



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